DRAFT
Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures

U.S. Department of Health and Human Services
Public Health Service
Agency for Toxic Substances and Disease Registry
Division of Toxicology

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found. The Food Quality Protection Act (FQPA) of 1996 requires that factors to be considered in establishing, modifying, or revoking tolerances for pesticide chemical residues shall include the available information concerning the cumulative effects of substances that have a common mechanism of toxicity, and combined exposure levels to the substance and other related substances. The FQPA requires that the Administrator of the Environmental Protection Agency (EPA) consult with the Secretary of the Department of Health and Human Services (which includes ATSDR) in implementing some of the provisions of the act.

To carry out these legislative mandates, ATSDR's Division of Toxicology (DT) has developed and coordinated a research program for chemical mixtures that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development. These efforts are interrelated. For example, the trend testing suggests mixtures of concern for further research, the mixtures toxicological testing contributes to the design and calibration of the models and validation of the methodology, and the modeling and methodology efforts suggest further testing to resolve issues and enhance understanding.

In this manner, ATSDR scientists, in collaboration with mixtures risk assessors and laboratory scientists, have been evolving an approach to the assessment of the joint toxic action of chemical mixtures over a number of years. This body of work, including published articles and book chapters, government documents, meeting reports, and unpublished reports, is the foundation of this document.

The public comment period ends on September 2, 2002. Comments should be sent to:

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THE GUIDANCE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEW:

Agency-wide review.

EXPERT PANEL REVIEW

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These experts collectively have knowledge of experimental, statistical, and modeling methods for mixtures, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in this document. A listing of the reviewers' comments, with a brief explanation regarding their inclusion or the rationale for their exclusion, exists as part of the administrative record for this document.

The citation of the expert panel review should not be understood to imply its approval of the document's final content. The responsibility for the content of this document lies with the ATSDR.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
BINWOE	Binary weight-of-evidence
BMD	benchmark dose
BTEXs	benzene, toluene, ethylbenzene and xylenes
CDD	chlorinated dibenzo-*p*-dioxin
CDF	chlorinated dibenzofuran
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DNA	deoxyribonucleic acid
DT	Division of Toxicology
EMEG	environmental media evaluation guide
EPA	Environmental Protection Agency
FQPA	Food Quality Protection Act
HI	hazard index
HQ	hazard quotient
IRIS	Integrated Risk Information System
ISS	Integral Search System
kg	kilogram
LOAEL	lowest-observed-adverse-effect level
mg	milligram
MRL	Minimal Risk Level
NAS	National Academy of Science
NIEHS	National Institute for Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PBPK/PD	physiologically based pharmacokinetic/pharmacodynamic
PCB	polychlorinated biphenyl
PEL	permissible exposure limit
ppm	parts per million
QSAR	quantitative structure-activity relationship
RfC	Reference Concentration
RfD	Reference Dose
SAR	structure-activity relationship
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
TLV	threshold limit value
TTD	target-organ toxicity dose
UF	uncertainty factor
U.S.	United States
WOE	weight-of-evidence

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to

EXECUTIVE SUMMARY

The *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures* (Mixtures Guidance Manual) is intended to assist environmental health scientists and toxicologists of ATSDR's Division of Toxicology (DT) in determining whether exposure to chemical mixtures at hazardous waste sites may impact public health. It serves a basis for interaction profiles, as the basis for health assessments and health consultations.

The ATSDR approach outlined in the Mixtures Guidance Manual is consistent with the approach articulated by EPA in 1986 and used to some extent, formally or informally, by a number of agencies. The approach is grounded in the law (CERCLA and the Food Quality Protection Act), and affords greater assurance of protection against adverse health effects than does the assessment of each chemical separately. The Expert Peer Review Panel, assembled on May 30-31, 2000 (see page iii), strongly approved of ATSDR's efforts to provide guidance for assessing joint toxic action of chemical mixtures and endorsed the ATSDR approach presented herein, which incorporates their comments and recommendations. The Mixture Guidance Manual also underwent ATSDR agency-wide review and incorporates comments received from these reviewers.

This guidance is designed to be used in conjunction with the ATSDR Public Health Assessment Guidance Manual, which provides the primary guidance for public health assessment, including aspects not covered in the Mixtures Guidance Manual. These additional aspects include exposure assessment guidance, recommended sources of health guideline values and toxicological information, and evaluation of health implications of other medical and toxicological factors, sensitive subpopulations, uncertainties, and community-specific health outcome data and community health concerns. The outcome of the public health assessment process is a determination of the category of public health hazard (ranging from urgent to no apparent public health hazard), and of follow-up actions including actions to protect public health, collection of additional health or site-characterization information, and community health education.

The systematic method outlined in the Mixtures Guidance Manual integrates ATSDR's interaction profiles, toxicological profiles, and research on chemical mixtures into a practical screening approach for potential health hazards. The conclusions from this exposure-based screening assessment of mixture hazard can then be taken into account *along with* biomedical judgment, the community-specific health outcome data, and community health concerns, to determine the public health implications and follow-up activities for a hazardous waste site.

The Mixtures Guidance Manual is organized so that the first three chapters provide background information considered important in understanding the ATSDR approach to mixtures assessment. The fourth chapter presents the ATSDR approach to exposure-based assessment of the joint toxic action of chemical mixtures. This approach is a semi-quantitative screening process. A step-by-step procedure is outlined in a flow chart for the assessment of noncarcinogenic effects and discussed in the accompanying text, followed by a series of examples illustrating the strategy. The strategy for the assessment of carcinogenic effects is then presented in a similar manner, with a flow chart, discussion, and series of examples.

The strategies for noncancer and cancer effects are similar. Exposure data and toxicological information on the mixture of concern (or a similar mixture) are the preferred basis for an assessment. If available, toxicological information on mixtures of concern for hazardous waste sites are likely to be reviewed and evaluated in ATSDR documents, including interaction profiles and toxicological profiles. If specific ATSDR documents or comparable documents from other agencies are not available, or do not provide Minimal Risk Levels (MRLs) or comparable health guideline values for the mixture or guidance regarding a health assessment approach, and if suitable whole mixture studies are not available, a components-based approach is undertaken.

The components-based approach focuses on mixture components that are present at toxicologically significant exposure levels, based on estimated exposures and relevant health guideline values. Linked physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for two or more components, if available, may be used to predict the potential for interactions, or possibly for noncancer or cancer health effects from the mixture. The hazard index method is used to screen for noncancer health hazards from potential additivity of the components. Cancer risks for the components are summed to screen for health hazards from potential additivity of carcinogenic effects. A weight-of-evidence method is used to evaluate the potential impact of interactions on noncancer and cancer health effects.

Additional technical detail regarding the concepts of dose and response addition, and the methodology for evaluating potential interactions, is provided in the appendices.

1. OVERVIEW

The health assessment of hazardous substances is complicated by the reality that most toxicological testing is performed on single chemicals, but human exposures are rarely limited to single chemicals. Exposures resulting from hazardous waste sites generally involve more than one hazardous substance (ATSDR 1992; De Rosa et al. 1996; Hansen et al. 1998; Johnson and De Rosa 1995). In addition, people voluntarily expose themselves to a variety of pharmacologically active chemicals such as those in recreational drugs (alcohol and tobacco), medicines, and foods, and are involuntarily exposed to other chemicals, such as those in vehicle exhaust, drinking water, and in the workplace.

The focus of this guidance is the exposure-based assessment of joint toxic action of chemical mixtures associated with hazardous waste sites, but suggestions for the appropriate consideration of non-site-related exposures also are provided.

1.1. INTRODUCTION

The term chemical mixture is used as "shorthand" for the concept of multiple chemical exposure. Some chemical mixtures are *intentional*—they are manufactured products, such as pesticide formulations, gasoline, or laundry detergent. Other chemical mixtures are *generated*—they are byproducts of such processes as smelting, drinking water disinfection, fuel combustion, and cigarette smoking. The chemical mixtures of concern at hazardous waste sites often are *coincidental*—they consist of unrelated chemicals from different sources, deposited separately at the site, but having the potential to reach the same "receptor population" by their presence in or migration into the same medium (commonly groundwater), or through a combination of media and pathways. (A receptor population is a population that is exposed or potentially exposed through identified exposure routes to contaminants at an exposure point [ATSDR 1992]). These categories of mixtures describe how the mixture originated.

ATSDR and other agencies such as the Environmental Protection Agency (EPA), National Institute of Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), and American Conference of Governmental Industrial Hygienists (ACGIH) derive health criteria, guidelines, or regulations primarily for single chemicals and, occasionally, for intentional or generated mixtures. The health values for the mixtures generally are based on data for the mixture itself, studied as if it were a single chemical. These mixtures include polychlorinated biphenyls (PCBs), certain fuels and pesticides, coal tar volatiles, and coke oven emissions.

For mixtures that are made up of relatively heterogenous components, however, health guidelines or regulations based on data for the original mixture may not be particularly useful for some exposure scenarios. For example, immediately following a release of gasoline to soil, inhalation exposure to the more volatile components, especially the low molecular weight alkanes, may be a concern. Contamination of ground and surface water with the more soluble components, including the BTEXs (benzene, toluene, ethylbenzene, and xylenes) may occur over a period of weeks to years, possibly impacting drinking water. The less mobile constituents such as benzo[a]pyrene may tend to remain in the soil at the site of the original release for extended periods. Thus, receptor populations are likely to be exposed to subsets of the original chemicals, and to different proportions of these chemicals than in the complete mixture. Health criteria or regulations based on toxicological data for the original mixture may not be applicable to the actual exposures resulting from a release, because *mixtures change with time and distance from the original release site, due to the differential fate and transport of their components.*

1.2. SOME CONCEPTS AND DEFINITIONS

Another set of mixture categories is useful in assessing the joint toxic action of chemical mixtures; these categories include simple and complex mixtures. Mixture definitions used in assessing the consequences to human health of joint toxic action of chemical mixtures are provided in Table 1.

In the absence of data and health criteria for the mixture of concern or a sufficiently similar mixture, the approach recommended by ACGIH (2000), EPA (1986, 1989a, 1990, 1999), NIOSH (1976), and OSHA (1993, 2001) has been to use the exposure and health criteria for the individual components of the mixture. The process involves evaluation of whether the exposures or risks for the components can reasonably be considered as additive based on the nature of the health effects. In addition, EPA recommends an evaluation of whether toxicological interactions among the components are likely to result in greater (or lesser) hazard or risk than would be expected on the basis of additivity alone.

The concern for ATSDR in terms of public health is similar; toxicological interactions may increase the health hazard above what would be expected from an assessment of each component singly, or all components additively. A particular issue is whether a mixture of components, each of which is present at less than guidance concentrations, may be hazardous due to additivity, interactions, or both.

As mentioned above, toxicological interactions can either increase or decrease the apparent toxicity of a mixture relative to that expected on the basis of dose-response relationships for the components of the

Table 1. Definitions of Chemical Mixture Terms*

Mixture	Any combination of two or more chemicals, regardless of source and spatial or temporal proximity, that may jointly contribute to actual or potential effects in a receptor population.
Simple Mixture	A combination of a relatively small number of chemicals (no more than 10) that have been identified and quantified (e.g., the components of concern for a receptor population near a hazardous waste site may constitute a simple mixture).
Complex Mixture	A combination of so many chemicals that the composition of the mixture is not fully characterized, either qualitatively or quantitatively, and may be variable (e.g., cigarette smoke, diesel exhaust, gasoline).
Similar Mixtures	Mixtures having the same chemicals but in slightly different proportions or having most but not all chemicals in common and in highly similar proportions. Similar mixtures are expected to have similar fate, transport, and health effects (e.g., the jet fuel JP-5 from different sources).
Chemical Class	A group of chemicals that are similar in chemical structure and biological activity, and which frequently occur together in the environment, usually because they are generated by the same process, such as manufacturing or combustion (e.g., PCBs, chlorinated dibenzo-*p*-dioxins [CDDs]).
Components	The chemicals that make up a mixture.
Components of Concern	The chemicals in a mixture that are likely contributors to health hazard either because their individual exposure levels exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard.
Index Chemical	The chemical selected as the basis for standardization of toxicity of components in a chemical class (e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD] for the assessment of dioxin-like compounds; benzo[a]pyrene for the assessment of carcinogenic polycyclic aromatic hydrocarbons [PAHs]).
Indicator Chemical(s)	A chemical (or chemicals) selected to represent the toxicity of a mixture because it is characteristic, potent, and has adequate dose-response data (e.g., benzene has been suggested as an indicator chemical for gasoline).

*Modified from EPA 1986, 1990, 1999; Fay and Feron 1996; Hertzberg et al. 1999.

mixture. Table 2 provides definitions of terms used in describing the results of interactions studies. These are the definitions that will be used in this document; other definitions exist. Some of the terms, such as additivity, refer to the lack of interactions. Interactions are defined as deviations from the results expected on the basis of additivity. Ultimately, the various types of interaction and noninteraction can be sorted into three categories: greater-than-additive (synergism, potentiation), additive (additivity, no apparent influence), and less-than-additive (antagonism, inhibition, masking).

Table 2. Interactions Terminology[a,b]

Interaction	When the effect of a mixture is different from additivity based on the dose-response relationships of the individual components.
Additivity	When the effect of the mixture can be estimated from the sum of the exposure levels (weighted for potency) or the effects of the individual components.
No apparent influence	When a component which is not toxic to a particular organ system does not influence the toxicity of a second component on that organ system.
Synergism	When the effect of the mixture is greater than that estimated for additivity on the basis of the toxicities of the components.
Potentiation	When a component that does not have a toxic effect on an organ system increases the effect of a second chemical on that organ system.
Antagonism	When the effect of the mixture is less than that estimated for additivity on the basis of the toxicities of the components.
Inhibition	When a component that does not have a toxic effect on a certain organ system decreases the apparent effect of a second chemical on that organ system.
Masking	When the components produce opposite or functionally competing effects on the same organ system, and diminish the effects of each other, or one overrides the effect of the other.

[a]Where effect is incidence or measured response, and additivity commonly is dose or response additivity.
[b]Based on definitions in EPA (1990, 1999), Hertzberg et al. (1999), and Mumtaz and Hertzberg (1993).

The major mechanisms for toxicant interactions are direct chemical-chemical, pharmacokinetic, and pharmacodynamic mechanisms. Knowledge of these mechanisms for two-chemical (binary) mixtures and for classes of chemicals can support the prediction of interactions for new combinations of chemicals. Most of these mechanisms affect the internal concentrations of the toxicants or their active forms. Table 3 lists examples of these types of interactions, primarily for compounds of occupational and environmental concern. A more detailed discussion of mechanisms of interaction is provided in a related Agency document, the *Guidance for the Preparation of an Interaction Profile* (ATSDR 2001).

Table 3. Mechanistic Bases of Toxicological Interactions among Chemicals*

Basis of interaction	Examples	
	Synergism or potentiation	Antagonism or inhibition
Chemical-chemical	Formation of nitrosamines (which are carcinogenic) from noncarcinogenic nitrites and amines in the stomach (Klaassen 1996)	Ammonia, administered orally, acts as antidote by reacting with ingested formaldehyde to form hexamethylene-tetramine (Goldstein et al. 1974)
Pharmacokinetic		
Absorption	Neurotoxicity of EPN (*o*-ethyl-*o*-4-nitrophenyl phenylphosphonothioate) enhanced by aliphatic hydrocarbons due in part to increased dermal absorption (Abou-Donia et al. 1985)	Dietary zinc inhibits some aspects of lead toxicity in part by decreasing dietary lead absorption (Cerklewski and Forbes 1976)
Distribution	Increased neurotoxicity from increased lead levels in brain after treatment with disulfiram, due to formation of complex that readily distributes lead to brain (Oskarsson and Lind 1985; Oskarsson et al. 1986a, 1986b)	Selenium protects against cadmium toxicity by decreasing the concentration of cadmium in liver and kidney and by redistributing cadmium in the testes from the low to high molecular weight Cd-binding proteins (Chen et al. 1975)
Excretion	Decreased renal excretion of penicillin when co-administered with probenecid, potentiating its therapeutic effect (Levine 1973)	Arsenic antagonizes the effects of selenium in part by enhancing the biliary excretion of selenium (Levander and Argrett 1969)
Metabolism	Organophosphorous compounds (profenfos, sulprofos, DEF) potentiate the toxicity of fenvalerate and malathion by inhibiting esterase which detoxifies many pyrethroid insecticides and also malathion (Gaughan et al. 1980)	Selenium inhibits 2-acetylamino-fluorene-induced hepatic damage and tumorigenesis in part by shifting metabolism towards detoxification (ring hydroxylation) relative to metabolic activation (9N-hydroxyla-tion) (Marshall et al. 1979)
Pharmacodynamic		
Interaction at same receptor site (receptor antagonism) or target molecule	No examples expected	Atropine antagonizes organophosphate poisoning by blocking acetylcholine receptor sites (Goldstein et al. 1974; Klaassen 1996)
Interaction at different sites on same molecule	Tiazofurin and selenazofurin metabolites bind to different sites on inosine monophosphate dehydro-genase to synergistically inhibit its activity (Chou and Rideout 1991).	Antagonism of copper binding to DNA by other divalent cations (Sagripanti et al. 1991)
Interaction among different receptor sites or targets	Potentiation of hepatoxicity of carbon tetrachloride by chlordecone inhibition of hepatocellular repair (Mehendale 1994)	Opposing effects of histamine and norepinephrine on vasodilation and blood pressure (functional antagonism) (Levine 1973)

*Adapted from EPA (1990) and Mumtaz and Hertzberg (1993).

The literature on interactions is limited in its direct applicability to mixtures associated with hazardous waste sites. As of 1991, the majority of interactions studies on chemicals were in the form of studies of the acute lethality or hepatotoxicity of binary mixtures administered by gavage or intraperitoneal injection to experimental animals (Hertzberg and Durkin 1994; Mumtaz and Durkin 1992; Mumtaz and Hertzberg 1993). Many of these studies employed a sequential treatment protocol, in which a chemical that alters metabolism or physiology in a known manner was administered before the chemical of concern, in order to investigate the impact on the second chemical's toxicity. This study design provided data useful in elucidating the mechanism of action of the second chemical, but not so useful in understanding potential interactions involving low level, long-term simultaneous exposure to chemicals in drinking water, food, soil, and air. Because of these and other limitations, a weight-of-evidence approach to the assessment of interactions may be useful.

Recently, another option for assessing interactions has become available: PBPK/PD modeling of mixtures. Although such models are available for very few mixtures at present, this is an area of active research and is promising because it supports the exploration of a variety of exposure scenarios.

2. OPTIONS AND ISSUES FOR THE ASSESSMENT OF JOINT TOXIC ACTION OF CHEMICAL MIXTURES

In general, mixtures health or risk assessment focuses on three methods: use of data for the actual mixture of concern (also called the whole mixture), use of data for a similar mixture, or use of data on the components of the mixture. These methods are listed in order of preference.

2.1. MIXTURE OF CONCERN (WHOLE MIXTURE, ORIGINAL MIXTURE)

When exposure data and health effects data are available for the mixture of concern, use of this data has traditionally been the preferred approach (EPA 1986). Data on the mixture of concern are rarely available. When available, such data tend to be for complex mixtures that are considered a health hazard because they are generated in large quantities and are thought to cause adverse health effects. In addition, the exposures of concern generally occur at the source of the mixture. An example is coke oven emissions. Health effects data are also available on pesticides, many of which are mixtures, often of isomers or congeners along with degradation products. A series of studies initiated by the National Institute of Environmental Health Sciences (NIEHS), however, focused on a mixture of 25 groundwater contaminants commonly associated with hazardous waste sites (Yang 1994). A similar study conducted on pesticide and fertilizer contaminants reported some evidence of cytogenetic damage (Kligerman et al.

1993; Yang 1994). These studies might be suitable as the basis for a mixture MRL or health guideline value, but this potential application of the studies does not appear to have been investigated.

The advantage of using data on the mixture of concern is that any interactions among the components of the mixture should be represented by the health effects data for the whole mixture. Limitations of the use of whole mixture data include the uncertainties regarding the extent to which the mixture from the exposure assessment "matches" the mixture that is the basis for the health criterion, due to changes in mixture composition with time and distance from the release, and/or differences in the original mixture. Thus, for most exposure scenarios, the mixture of concern will likely not be identical to the mixture that is the basis for the health criterion, even when it is called by the same name (e.g., toxaphene, PCBs). Further guidance on this topic is provided in Step 2 in Sections 4.2.1 and 4.2.3 of this guidance, and in the ATSDR (2001) interaction profile guidance.

2.2. SIMILAR MIXTURE

If no adequate data are available on the mixture of concern, but health effects data or guidance values are available on a similar mixture, the risk or health assessment may be based on the health effects data for the similar mixture, if the mixtures are sufficiently similar (EPA 1986, 1999). Sufficiently similar mixtures are those having the same chemicals but in slightly different proportions, or having most but not all chemicals in common and in highly similar proportions. In addition, the mixtures and their components have similar fate, transport, and health effects, whereas insufficiently similar mixtures may not. For example, JP-5 from different sources is considered similar because it is produced to meet uniform specifications, and differences from one source to another are thought to be minor (ATSDR 1998a). Gasoline from different sources was not considered sufficiently similar because of the wide range of formulations (ATSDR 1995a; Pohl et al. 1997). In addition, gasoline is a relatively heterogeneous mixture whose components have widely differing fate and transport characteristics (ATSDR 1995a, 1999). Consequently, receptor populations are likely to be exposed to subsets of the original components, and the subsets (or fractions) are not sufficiently similar to the original mixture (see Section 1.1).

Another method that has been used for risk assessment of similar mixtures is the comparative potency method. In this procedure, data for a set of similar mixtures are used to estimate a scaling factor that relates cancer potency derived from a chronic animal study or human epidemiology study to potency in a simpler assay, such as a mouse skin painting study. Then the cancer potency factor for an additional similar mixture for which only data from the simpler assay are available can be estimated using this

scaling factor (Calabrese 1991; EPA 1999; Hertzberg et al. 1999; NRC 1988). This procedure has been used in the estimation of human cancer risk from combustion emissions from various sources (Albert et al. 1983; Lewtas 1985, 1988). Methods for noncarcinogenic effects are beginning to be developed (EPA 1999).

2.3. COMPONENTS

Due to the lack of suitable health criteria for the mixture of concern or a similar mixture, approaches involving the components of a mixture are commonly used for the incidental mixtures associated with hazardous waste sites. These methods are based on an assumption that the exposures or the responses to the mixture components are additive.

Dose Addition, also known as concentration addition, simple similar action, and similar joint action, assumes that the components of a mixture behave as concentrations or dilutions of one another, differing only in their potencies (Bliss 1939; Finney 1971). The dose-response curves are parallel (i.e., the regression lines of probits on log doses are parallel), and tolerance (or susceptibility) to the components is completely positively correlated (the organisms most susceptible to chemical A also will be most susceptible to chemical B). The response to the mixture can be predicted by summing the doses of the components after adjusting for the differences in potencies. Dose addition is considered most appropriate for mixtures with components that affect the same endpoint by the same mode of action (EPA 1986, 1990, 1999). It has been suggested that the requirement for parallel dose-response curves and complete correlation of tolerances may be too stringent (e.g., Plackett and Hewlett 1952; Svendsgaard and Hertzberg 1994), and that in the low-dose region in which the response is linear, dose additivity may hold for independently acting chemicals as well (Svendsgaard and Hertzberg 1994). Dose addition is the underlying assumption of the hazard index method and the toxic equivalency factor (TEF) approach (Sections 2.3.1 and 2.3.3).

Response Addition, also known as simple independent action and independent joint action (Bliss 1939), assumes that the chemicals act independently and by different modes of action. Tolerance (or susceptibility) to one component may or may not be correlated with tolerance to another. The organisms most susceptible to chemical A may also be most susceptible to chemical B (complete positive correlation) or may be least susceptible to chemical B (complete negative correlation), or the susceptibilities to the two chemicals may be statistically independent. The response to the mixture can be predicted from the responses to the components and the correlation of tolerances. Response addition is the underlying assumption of an approach to cancer risk assessment for mixtures and ACGIH's

approach to assessing the hazard of occupational exposure to agents that act independently (Sections 2.3.5 and 3.1).

Additional detail regarding dose and response addition is provided in Appendix A.

Evidence to Support the Use of Dose Additivity Models

Acute studies using overtly toxic doses of binary mixtures have shown that deviations from dose additivity generally are not remarkable in mammals (e.g., Smyth et al. 1969, 1970; Withey and Hall 1975). Toxicity studies on guppies and frogs using mixtures of 3 to as many as 50 components also tend to indicate that deviations from dose addition are not substantial (e.g., Dawson 1994; Hermens et al. 1985; Konemann 1981). Deviations from dose additivity were generally less than a factor of five. A number of investigations have focused on the low dose (low response) area. In a series of 4-week feeding studies by the TNO Nutrition and Food Research Institute, mixtures of four chemicals were administered orally to rats at doses of the individual chemicals below the no-observed-adverse-effect level (NOAEL), equivalent to the NOAEL, and at an adverse effect level. These studies gave results for renal toxicity that were consistent with dose additivity or that appeared less-than-dose-additive. The mixtures consisted of four similarly acting nephrotoxicants (Feron et al. 1995) and four dissimilarly acting nephrotoxicants (Jonker et al. 1993). The above conclusions are based partly on the investigators' observations, and partly on a reanalysis of the individual animal data using exponential dose-response functions, performed for ATSDR (Mumtaz et al. 1998). Results of other studies by the same institute on mixtures of eight (Jonker et al. 1990) and nine (Groten et al. 1997) dissimilarly acting chemicals reported few effects when the doses of the individual components of the mixture were subtoxic.

Other studies, however, indicate co-exposure to subthreshold doses or environmental doses of chemicals that affect the same target organs (though not by the same mechanism) can result in adverse effects. An acute study of a mixture of subthreshold doses of 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene in rats resulted in adverse effects on the liver (Stacey 1989). Although cadmium and lead affect the hematological system through different mechanisms, dietary exposures of rats to these metals at doses that did not significantly affect hemoglobin and hematocrit when given individually, resulted in significant decreases in hemoglobin and hematocrit when given as a mixture (Mahaffey and Fowler 1977; Mahaffey et al. 1981). A series of studies initiated by the NIEHS on a mixture of 25 groundwater contaminants from hazardous waste sites indicated that toxic effects can result from long-term exposure to mixtures in which each of the components is present at doses expected to be subtoxic (Yang 1994). A similar NIEHS study conducted on pesticide and fertilizer contaminants

reported some evidence of cytogenetic damage (Kligerman et al. 1993; Yang 1994). The individual components were not tested in the NIEHS studies, so further analysis of the data for interactions or additivity is problematic, but the authors used doses of the individual chemicals that were expected to be without effect. Epidemiological studies of children have indicated that lead and arsenic, and lead and cadmium, may interact at environmental levels of exposure to produce adverse neurobehavioral consequences in children (Marlowe et al. 1985; Moon et al. 1985). On the other hand, some studies in animals and humans (e.g., Berman et al. 1992; Caprino et al. 1983; Drott et al. 1993; Harris et al. 1984) have reported apparent thresholds for interactions (see also Section 2.3.7).

Data that indicate a lack of interactive effects may not, however, mean there is no interaction. The biological systems currently in use may not be sensitive enough to detect interactions, and the power of many joint toxic action studies may be insufficient to conclusively demonstrate additivity or interactions. Newer techniques, such as genomics and proteomics, may provide tools for detecting toxicological interactions at very low dose levels.

Based on the above evidence and concerns, the dose-additivity assumption may be a reasonable *default* assumption for chemicals with similar effects or the same target organ in the low dose range. Use of the dose-additivity assumption is likely to produce estimates of health hazard that range from appropriate to somewhat conservative, and which are therefore protective of public health.

2.3.1. Hazard Index

The hazard index approach uses the assumption of dose additivity to assess the noncancer health effects of a mixture from the data on the components. EPA has adopted the term "hazard index" for this approach, which appears to have originated in 1972 (see Section 3.5). The approach is used or recommended by a number of agencies (ACGIH 2000; EPA 1986, 1989a; Mumtaz et al. 1994a, 1997; National Academy of Sciences [NAS] 1974; National Research Council [NRC] 1989; OSHA 1993, 2001). Exposures or doses for the various components of the mixture are scaled by a defined level of exposure generally regarded as "acceptable" or "safe" by the agency performing the assessment. The defined levels could be ATSDR MRLs, EPA reference doses (RfDs) or reference concentrations (RfCs), ACGIH threshold limit values (TLVs), or OSHA permissible exposure limits (PELs). The general equation for the hazard index (*HI*) is:

$$(a) \quad HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \cdots + \frac{E_n}{DL_n} \qquad or \qquad (b) \quad HI = \sum_{i=1}^{n} \frac{E_i}{DL_i} \qquad (1)$$

In equation 1(a), E_1 is the level of exposure to the first chemical in the mixture and DL_1 is some defined level of exposure to the first chemical, E_2 and DL_2 are the corresponding levels for chemical 2, and the summation can extend to any number of chemicals, signified by the n. Equation 1(b) simply expresses the same idea more succinctly, where i is the i^{th} chemical. Each chemical-specific ratio (e.g., E_1 / DL_1) is called a hazard quotient (HQ). Therefore, the hazard index can be expressed as the sum of the hazard quotients:

$$HI = \sum_{i=1}^{n} HQ_i \qquad \textbf{(2)}$$

When the hazard quotient for a single chemical exceeds unity, concern for the potential hazard of the chemical increases. Similarly, when the hazard index for a mixture exceeds unity, concern for the potential hazard of the mixture increases.

Separate hazard indexes are estimated for each pathway and exposure duration of concern. For a given duration, hazard indexes are summed across pathways that affect the same receptor population.

The obvious advantage of this method is its simplicity. Because it is based on the assumption of dose additivity, the hazard index method is most appropriately applied to components that cause the same effect by the same mechanism of action. In practice, it may be applied to components with different target organs (sometimes as a screening measure). The method is frequently applied to components with the same critical target organ or critical effect (effect that is the basis for the MRL, RfD, or other health guideline), without regard to mechanism of action. For Superfund risk assessments, strong evidence is required to indicate that two compounds producing adverse effects on the same organ system, although by different mechanisms, should not be treated as dose additive (EPA 1989a). See also the discussion in Section 2.3 (*Evidence to Support the Use of Dose Additivity Models*).

The hazard index method does not take into account interactions among the components of the mixture.

Additional information on this method is provided in EPA (1986, 1989a).

2.3.2. Target-organ Toxicity Dose Modification to Hazard Index Method

The target-organ toxicity dose (TTD) method, which is a refinement of the hazard index method, was devised in order to accommodate the assessment of mixtures whose components do not all have the same critical effect. In addition, it takes into account the reality that most components of waste-site-related mixtures affect other target organs at doses higher than those that cause the critical effect. These other

effects may vary from component to component and may be important in assessing the health effects of the mixture. EPA (1989a) suggested that separate hazard indexes be estimated for all endpoints of concern. EPA further suggested that the RfD be used not only in generating hazard quotients for the critical effect of a component, but also in estimating hazard quotients for effects that occur at higher exposure levels. As acknowledged by EPA (1989a) and demonstrated by Mumtaz et al. (1994a, 1997), this practice may overestimate the hazard for effects occurring at exposure levels higher than those associated with the critical effect. The use of TTDs was therefore suggested (Mumtaz and Colman 1992; Mumtaz et al. 1997), and is consistent with the recommendations of EPA (1986) and NRC (1989), discussed in Sections 3.4 and 3.5. TTDs are developed for the chemicals that affect an endpoint at a dose higher than that for the critical effect for the same chemical. A TTD for each endpoint of concern is calculated using appropriate MRL (or RfD) methodology, and then used in estimating the endpoint-specific hazard indexes. The MRL (or RfD) is used for the critical effect for each chemical and the TTD is used for the other endpoints of concern for the chemical. When any of the endpoint-specific hazard indexes exceeds unity, concern for the potential hazard of the mixture increases.

The derivation of TTDs for use in assessment of the joint toxic action of chemical mixtures is analogous to the derivation of MRLs, and should follow the applicable portions of ATSDR (1996a) MRL guidance. TTDs are based on the other major characteristic effects of a chemical, which are known to occur at the same or higher exposure levels as the critical effects. Like the derivation of an MRL, the derivation of a TTD is not recommended for an endpoint that is affected only at the relatively high levels of exposure associated with severe effects. Because the purpose of TTD derivation is to support the estimation of endpoint-specific hazard indexes (Guidance Manual Section 2.3.1; Mumtaz et al. 1994a, 1997), TTD derivations should be performed for endpoints that are common to more than one component of a given mixture. In addition, endpoints identified as concerns in populations exposed to the mixture should be considered.

In common with MRLs, TTDs are specific for route and exposure period. The TTD should be based on the highest NOAEL that does not exceed a lowest-observed-adverse-effect level (LOAEL) for the particular endpoint, as determined from the information in toxicological profiles, including the Levels of Significant Exposure Tables. If such a NOAEL is not available, the TTD would be based on the lowest LOAEL for that endpoint. Additional considerations, as for MRL derivation, are that the NOAEL or LOAEL used as the basis for the TTD should be from a representative, quality study, for the same route and exposure period as the TTD. When data for the exposure duration of concern are not available, a TTD derived for one duration may sometimes be applicable for other duration(s) of the same route, if supported by the overall database. An additional uncertainty factor may be applied to extrapolate across

exposure durations, based on scientific judgment. Dose adjustments and interspecies, intraspecies, and LOAEL to NOAEL extrapolation should be performed as for an MRL. When suitable data are available, and when appropriate, TTDs can also be derived using benchmark dose (BMD) modeling (Crump 1984, 1995; EPA 2001; Gaylor et al. 1998) to define the BMD, which is used in place of a NOAEL as the basis for TTD derivation, similar to the procedure for MRL derivation.

For example, suppose that chemicals 1, 2, 3, and 4 are commonly found in combination in completed exposure pathways involving intermediate oral exposure. The intermediate oral MRLs for chemicals 1 and 2 are based on hepatic effects, and for chemicals 3 and 4 are based on renal effects and developmental effects, respectively. Each of these endpoints also is affected by at least one other mixture component for which it is not the critical effect. Other major effects in common for two or more of these chemicals for this route and duration include neurological and reproductive effects. In addition, chemical 1 causes immunological effects and chemical 4 causes endocrine (adrenal) effects during intermediate oral exposure. At levels of exposure that cause high mortality, chemical 1 also causes hematological effects in rats. This information is summarized in Table 4.

Table 4. Endpoints Affected by Chemicals 1, 2, 3, and 4

	AFFECTED BY			
ENDPOINT	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Hematological	with mortality	No	No	No
Hepatic	Yes—MRL	Yes—MRL	No	Yes
Renal	Yes	No	Yes—MRL	Yes
Endocrine (adrenal)	No	No	No	Yes
Immunological	Yes	No	No	No
Neurological	Yes	Yes	Yes	No
Developmental	Yes	Yes	Yes	Yes—MRL

The endpoints of concern chosen for TTD derivation, based on the critical effects of the chemicals and on other major effects in common for this set of chemicals, are hepatic, renal, neurological, and developmental effects. These endpoints are shown in bold italicized print in the table. Since adrenal and immunological effects each are caused by only one chemical, and are not the critical effects for any of the components of the mixture, the estimation of endpoint-specific hazard indexes is not needed for these

endpoints, and TTDs are accordingly not developed. For a different mixture of chemicals that included chemical 1, the immunological endpoint may warrant TTD derivation if at least one other chemical in the mixture also causes this effect. Similar reasoning would apply for chemical 4 and adrenal effects. The hematological effects are not a suitable basis for TTD derivation for chemical 1 not only because they are caused by only one chemical, but also because they occurred only at levels of exposure that caused significant mortality.

For the purposes of illustration, a TTD for renal effects will be derived for chemical 1. The intermediate oral MRL for chemical 1 is 0.15 mg/kg/day based on a NOAEL of 15 mg/kg/day for hepatic effects in experimental animals given the chemical orally for an intermediate duration. The NOAEL was divided by an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability) to estimate the MRL. The LOAEL for hepatic effects in the same study was 30 mg/kg/day. The NOAEL and LOAEL for renal effects in the this study were 30 and 45 mg/kg/day, and were the most reliable data for this effect. In addition, the NOAEL was the highest NOAEL for this effect. A TTD_{RENAL} of 0.3 mg/kg/day is derived by dividing the $NOAEL_{RENAL}$ of 30 mg/kg/day by an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability). Derivation of TTDs for the other effects would proceed in a similar manner.

Following derivation of the TTDs, endpoint-specific hazard indexes are calculated as follows:

$$
\begin{aligned}
(a) \quad HI_{HEPATIC} &= \frac{E_1}{MRL_1} + \frac{E_2}{MRL_2} + \frac{E_4}{TTD_{4HEPATIC}} \\
(b) \quad HI_{RENAL} &= \frac{E_1}{TTD_{1RENAL}} + \frac{E_3}{MRL_3} + \frac{E_4}{TTD_{4RENAL}} \\
(c) \quad HI_{NEURO} &= \frac{E_1}{TTD_{1NEURO}} + \frac{E_2}{TTD_{2NEURO}} + \frac{E_3}{TTD_{3NEURO}} \\
(d) \quad HI_{DEV} &= \frac{E_1}{TTD_{1DEV}} + \frac{E_2}{TTD_{2DEV}} + \frac{E_3}{TTD_{3DEV}} + \frac{E_4}{MRL_4}
\end{aligned}
\qquad (3)
$$

where $HI_{ENDPOINT}$ is the hazard index for indicated endpoint (*HEPATIC*, *RENAL*, *NEURO* [neurological], *DEV* [developmental]), E_i is the exposure for the i^{th} chemical (1, 2, 3, or 4 in the above example), MRL_i is the MRL for the i^{th} chemical, and TTD_i is the TTD for the i^{th} chemical for the indicated endpoint. (If an MRL is not available, a suitable RfD can be used.) Although developmental toxicity is the critical effect for only one of the four chemicals, all four produce the effect, and it is conceivable that it may be a sensitive effect for the mixture. Neurological effects are not the critical effect for any of the chemicals, but three of the chemicals cause this effect at equivalent or higher exposure levels than associated with the critical effect. Thus, use of the TTD modification of the hazard index for mixtures of chemicals that

do not have the same critical effect may increase the understanding of the potential impact of the mixture on public health. Additional information regarding this method is provided by Mumtaz et al. (1994a, 1997).

The development of TTDs is analytically intensive. TTDs have been developed for a variety of chemicals in a pilot study (Mumtaz et al. 1997) and are being developed in ATSDR interaction profiles. The derivations in the interaction profiles are subjected to a review process that is similar to that for MRLs. The development of these values for all substances that are currently the subjects of toxicological profiles, for each duration and route of exposure, would be problematic. To address the issue of practicality, the method could be limited to those situations where clarification of the public health hazard is needed (as described in Sections 4.2.2 and 4.2.3), the TTD effort could be focused on chemicals that frequently occur together in mixtures of concern needing such clarification of public health hazard, and TTD determinations could be made available to health assessors through an easily accessible and readily updated medium, such as the ATSDR website, or through interaction profiles. If the method proves useful, the addition of TTDs to the toxicological profiles could be considered, to be phased in as new profiles are developed and existing profiles are updated. The TTDs could be developed and reviewed in conjunction with MRLs.

2.3.3. Weight-of-Evidence Modification to the Hazard Index

As noted above, the hazard index method does not incorporate information on interactions among components of the mixture. A weight-of-evidence (WOE) method proposed by Mumtaz and Durkin (1992) was the first systematic attempt to address this need. The method implemented and expanded on the suggestion made by the NRC (1989) that, in recognition of the difficulties of quantifying interactions, an uncertainty factor (UF) be used to account for interactions among components of a mixture (Section 3.5). The method was designed to modify the hazard index to account for interactions, using the weight of evidence for interactions among pairs of mixture components. Although subsequent experience with the algorithm used to generate the interactions hazard index has revealed that it does not handle changes in proportions of mixture components in a reasonable manner, the method is useful qualitatively for predicting whether hazard may be greater or less than indicated by the hazard index.

The method evaluates data relevant to joint action for each possible pair of chemicals in the mixture in order to make qualitative binary weight-of-evidence (BINWOE) determinations for the effect of each chemical on the toxicity of every other chemical. Two BINWOEs are needed for each pair: one for the effect of chemical A on the toxicity of chemical B, and another for the effect of chemical B on the

toxicity of chemical A. The BINWOE determination is a classification that indicates the expected direction of an interaction (greater than additive, less than additive, additive, or indeterminate), and scores the data qualitatively, using an alphanumeric scheme that takes into account mechanistic understanding, toxicological significance, and relevance of the exposure duration, sequence, bioassay (*in vitro* versus *in vivo*), and route of exposure. The alphanumeric terms in the classification scheme can then be converted to a single numerical score, by multiplying the corresponding direction factor by the data quality weighting factor. Although the earlier publications of the WOE method did not discuss the need for BINWOE determinations to take into account target organ (Durkin 1995; Mumtaz and Durkin 1992), experience in application of the WOE method, including preparation of the ATSDR interaction profiles and a study by Mumtaz et al. (1998), has indicated that the WOE evaluations should be target-organ specific.

The qualitative BINWOE classifications are shown in the left column of Table 5 and the direction factors and data quality weighting factors are shown in the far right column. An alphanumeric (qualitative) BINWOE classification of >II.B.2.a.i for the effect of one chemical on the toxicity of another thus corresponds to greater-than-additive interaction, mechanistic data on related chemicals, inferred toxicological significance, different duration or sequence, *in vivo* data, and anticipated route of exposure. The corresponding BINWOE score is +1(0.71)(0.71)(0.79)(1)(1)=+0.40.

The weight of evidence method used the numerical BINWOE scores as the interaction terms in an equation that took into account the doses and potencies (through use of hazard quotients) of the components of the mixture, and calculated a composite score for interactions, WOE_N, that was intended to be an expression of the strength of the evidence that interactions may be toxicologically significant relative to the highest possible level of certainty that would be possible for the particular mixture. Details are provided in Appendix B. The WOE_N was used to modify an interactions uncertainty factor (UF_I), as follows:

$$HI_I = HI_{add} \times UF_I^{WOE_N} \quad \textbf{(4)}$$

where HI_I is the interactions-adjusted hazard index and HI_{add} is the hazard index based on additivity. An uncertainty factor of 10 was chosen in exercises illustrating the method (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). Because this algorithm does not does not handle changes in proportions of mixture components in a reasonable manner, a qualitative WOE method is used, as described in the following paragraph.

Table 5. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

	Classification	Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	-1
?	Indeterminate	0
Quality of the Data		**Weighting**
	Mechanistic Understanding	
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur have not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
	Toxicological Significance	
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
	Modifiers	
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79
Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05		
BINWOE = Direction Factor x Weighting Factor: Ranges from –1 through 0 to +1		

*Adapted from Mumtaz and Durkin (1992) and Mumtaz et al. (1994a)

A qualitative WOE approach, focusing on application of the BINWOE scores to hazardous waste-site assessment, was suggested by Mumtaz and Durkin (1992). This approach is appropriate for a mixture where the scaled doses (hazard quotients) for all of the components are similar, or toxicologically significant. The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2-N)/2$. Thus, this mixture of 4 components has 6 pairs of components and potentially 11 BINWOEs. Suppose nine of the BINWOEs are greater-than-additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the weight of evidence suggests that the mixture is likely to pose a greater hazard than that indicated by the hazard index.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater than additive, some less than additive, and some additive BINWOEs). In this case, the qualitative WOE approach is extended to include conversion of the qualitative BINWOE scores to numerical scores, and summing the scores to give a combined score. If the combined BINWOE score is positive and significantly different from zero, then the weight of evidence suggests that the mixture is likely to pose a greater hazard than indicated by the hazard index. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the weight of evidence suggests that the health hazard is unlikely to be greater than indicated by the hazard index. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index.

Although the WOE method was developed for assessing interactions for noncarcinogenic effects, the qualitative WOE method is equally applicable to assessing interactions for carcinogenic effects.

The WOE method (Mumtaz and Durkin 1992; Mumtaz et al. 1994a) has undergone evaluation, and appeared to perform well qualitatively, and quantitatively under some circumstances. The application of the method for deriving BINWOE classifications was considered consistent by expert toxicologists who reviewed the results of exercises in which several teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals (Mumtaz et al. 1994b). In tests of the WOE method to predict the toxicity of some simple chemical mixtures to animals, BINWOEs for three pairs of chemicals qualitatively predicted whether the results of animal studies would be less-than-additive, additive, or greater-than-additive (Mumtaz et al. 1998). Used with an exponential dose-response model and dose addition to model relative kidney weights, the quantitative WOE method closely predicted the observed dose-response in female rats for intermediate-duration oral exposure to a

mixture of four nephrotoxic chemicals with similar modes of action (Mumtaz et al. 1998). The observed dose-response was less than dose additive. The BINWOEs were focused on renal toxicity, and the uncertainty factor used in the algorithm was 10. The WOE method underestimated the relative liver weights in the same animals. The observed dose-response for relative liver weight was slightly greater than dose additive. Thus, the WOE method did not predict toxicity to a target organ that was different from the one for which the BINWOEs were derived. The WOE method slightly overpredicted the observed dose-response for relative kidney weight in male rats for a mixture of dissimilarly acting nephrotoxins (in female rats, the data variability was so great that the exponential model did not fit the *observed* responses) (Mumtaz et al. 1998). Although these results are suggestive, limitations of this test of the complete WOE method include the substantial variability in the responses of individual animals, small numbers of animals per group, testing of only two dose levels of the mixtures, and lack of rationale for using relative organ weight as an index of toxicity (several other indicators of renal and hepatic toxicity were monitored in the studies that provided the experimental data [Jonker et al. 1993, 1996]).

A modification of the original WOE method was proposed by Eastern Research Group (ERG) and Durkin (1995) and has been further developed by EPA and adopted as part of its mixtures guidance (EPA 1999). This modification includes a slightly different classification scheme and a different method of calculating the interactions-modified hazard index. The method encourages greater use of quantitative interaction data through the use of magnitude-of-interaction factors for each chemical pair. The classification scheme, while more integrated in nature, requires more judgment, and the type of quantitative interaction data required to estimate the magnitude factor is rarely available. The algorithm for this modification appears to handle changes in proportions of mixture components more reasonably than does the original algorithm, but additional evaluation with regard to predicting experimental results is desirable.

A basic assumption of both WOE methods is that interactive interference will not be significant. For example, if chemicals A and B interact in a certain way, the presence of chemical C will not cause the interaction to be substantially different. Thus, the assumption is that pairwise interactions will dominate in the mixture and adequately represent all the interactions.

Additional detail regarding both methods is provided in Appendix B, and detailed guidance for deriving BINWOE determinations and evaluating joint toxic action studies is presented in ATSDR (2001).

2.3.4. Toxic Equivalency and Relative Potency

The toxic equivalency and relative potency approaches also use the assumption of dose additivity to assess the health effects of a mixture. These approaches have been applied to mixtures that consist of a class of chemicals, and are used when health effects information for one component of the mixture is sufficient to derive health criteria but for the other components of the mixture is less complete.

The toxic equivalency approach has been used with the CDDs and structurally related chemical classes such as the chlorinated dibenzo-*p*-furans (CDFs) and the coplanar PCBs (Ahlborg et al. 1994; ATSDR 1998b; EPA 1989b, 1994; Safe 1998; Van den Berg et al. 1998). This method estimates toxic equivalency factors (TEFs) for the various congeners in the mixture based on the key assumption that certain congeners exert effects such as carcinogenicity through a common receptor-mediated mechanism (Ah receptor), and therefore act in a dose additive manner. The TEF approach compares the potency of individual congeners, based on *in vitro* or acute *in vivo* data, with that of 2,3,7,8-TCDD, the best-studied of this chemical class. 2,3,7,8-TCDD is assigned a TEF of unity; the other TEFs (or relative potencies) are usually less than one. The concentrations or doses of each active congener are multiplied by their TEF values and then summed to give the total toxic equivalents (TEQs) of a mixture:

$$TEQs = \sum_{i=1}^{n} C_i \times TEF_i \qquad \textbf{(5)}$$

where C_i is the concentration (or dose) and TEF_i is the TEF for the i^{th} component of the mixture. The TEQ thus represents the concentrations of all the components as an equivalent concentration of the index chemical, 2,3,7,8-TCDD. The hazard or risk of exposure to the mixture is estimated by comparing the TEQs with MRLs or other health-based criteria (ATSDR environmental media evaluation guide [EMEG]; ATSDR screening, evaluation, and action levels) based on 2,3,7,8-TCDD (ATSDR 1998b; De Rosa et al. 1997a, 1998; Mumtaz and Hertzberg 1993; Pohl et al. 1995) or multiplying the TEQ (in appropriate units of mg/kg/day or mg/m^3) by a cancer slope factor or unit risk for 2,3,7,8-TCDD (EPA 1994, 1996; Mumtaz and Hertzberg 1993).

This approach is considered suitable for the assessment of health effects of dioxin-like compounds that are mediated through the Ah receptor, but is not applicable for those that are not (ATSDR 1998b). Carcinogenicity (at least in part), immunotoxicity, and developmental and reproductive toxicity (the basis for oral MRLs) are thought to be mediated through the Ah receptor (ATSDR 1998b). Limitations to this method are that some of the nondioxin-like PCB congeners have been shown to inhibit or enhance responses to 2,3,7,8-TCDD, depending on dose and assay system (Birnbaum and DeVito 1995; Pohl and Holler 1995; Safe 1998); the range of TEF values estimated for some PCB congeners is very broad (Safe

1998); and a slope factor for 2,3,7,8-TCDD is not available on the Integrated Risk Information System (IRIS). The TEF approach continues to evolve and undergo additional testing and validation. ATSDR considers the approach less suitable for PCBs, and has derived MRLs for PCBs (ATSDR 2000). ATSDR is using the TEF method as a tool for assessing health effects of dioxin and dioxin-like compounds (primarily CDDs and CDFs) in soil (ATSDR 1998b; De Rosa et al. 1997a, 1998).

A similar approach, called a relative potency approach, has been developed for PAHs that have been classified as B2 carcinogens by EPA (ATSDR 1995b; EPA 1993). The relative potency factors are estimated on the basis of potency relative to that of benzo[a]pyrene in skin painting carcinogenesis studies. Benzo[a]pyrene is the best-studied of this class and has a cancer potency factor available on IRIS. The mechanistic underpinnings of the relative potency approach for the PAHs are less good, in terms of the additivity assumption. Some of the same issues as noted for the application of the TEF approach also are issues for the use of the relative potency method for PAHs, including nonadditive interactions among the PAHs.

2.3.5. Total Cancer Risk

A response addition approach has been recommended for the assessment of risk from mixtures of carcinogenic chemicals (De Rosa et al. 1993; EPA 1986, 1999; Mumtaz et al. 1994a; NRC 1989). The most conservative form of response addition, completely negative correlation of tolerances (i.e., individuals most sensitive to chemical A are least sensitive to chemical B and vice versa; see Appendix A) was recommended by EPA (1986). Accordingly, the response or risk for the mixture is the sum of the risks for the components:

$$Risk = \sum_{i=1}^{n} Risk_i = \sum_{i=1}^{n} d_i B_i \qquad (6)$$

where $Risk_i$ is the risk, d_i is the dose, and B_i is a potency parameter (slope factor or unit risk) for the i^{th} carcinogen. The equation is appropriate when risks for the individual chemicals are less than 0.01 and the sum of the individual risks is less than 0.1 (EPA 1989a). This equation is equivalent to dose addition if the dose-response curves for the chemicals are within the linear (low-dose) range, and have no threshold (EPA 1986, 1999). EPA (1999) recommends the response addition model for independent action (as in equation 18 of Appendix A) for cancer risk, noting that when component risks are small, the formula collapses to the simple addition of component risks (equation 6 above). Use of the IRIS values for slope factor or unit risk result in plausible upper bounds to the lifetime excess cancer risk of the components. Concern has been raised that summing upper bound risks may lead to unreasonably high estimates of the mixture risk, but an analysis by Kodell and Chen (1994) suggests that the error in the

simple sum of the upper bound risks is small relative to other uncertainties, and Cogliano (1997) concluded that the sum of the upper bound risks provides useful information regarding the overall risk from mixtures of carcinogens.

2.3.6. The Integral Search System (ISS) for Ranking Hazards of Mixtures of Carcinogens

The ISS method (Woo et al. 1994), like the WOE method, uses data for binary mixtures to predict the hazard of exposure to mixtures of three or more chemicals. The method is carried out by a software package. The ISS integrates three EPA and National Cancer Institute databases on binary interactions of carcinogens with carcinogens, promoters, and inhibitors. It contains approximately 1,000 chemicals of 60 structural and functional classes. The ISS calculates a weighting ratio reflecting the ratio of greater-than-additive to less-than-additive interactions for the components of a mixture. The estimation of the weighting ratio is based on the interactions data for the chemical pairs in the mixture and, for those pairs lacking interactions data, on interactions between other members of the chemical classes to which the chemicals belong. The weighting ratio also incorporates judgments as to the relative effectiveness of the four type of interactions (synergism, promotion, antagonism, and inhibition) in modifying the hazard. Weighting ratios greater than unity indicate that the combined effect of the mixture components is expected to be greater-than-additive, whereas ratios less than unity indicate that the combined effect is expected to be less-than-additive.

In addition, ISS can be used to estimate a "concern level," which is based on the "inherent hazard" (the sum of the slope factors for the components, converted to an exponent index value), multiplied by the weighting ratio. The resulting score is converted back to a weighted total slope factor and to a corresponding concern level, ranging from low to high.

A serious limitation, however, is that ISS does not include exposure concentration or dose as part of this procedure. Another serious limitation is that the class-class interaction ratings for pairs of chemicals with no data tend to dominate the score. The attractive features of the ISS are that it calculates the weighting ratios automatically, it is applicable to mixtures with relatively large numbers of components, and it can accommodate the assessment of chemicals that are not presently included in the database as long as the chemical can be assigned to an appropriate class of chemicals within the database. Additional detail regarding ISS is provided in Appendix C.

2.3.7. PBPK, PBPK/PD, and Quantitative Structure Activity Relationships (QSAR)

PBPK and PBPK/PD techniques are beginning to be applied to problems in mixtures toxicology. For mixtures of two chemicals, PBPK and PBPK/PD models for the individual chemical are linked at the assumed point of interaction, frequently the hepatic metabolism term. Following validation of the assumed mechanism by comparing model predictions with experimental data, the model can be used to predict effects of co-exposure for different exposure scenarios. For example, binary PBPK models have been developed to extrapolate from high-exposure inhalation studies of interactions of toluene and xylene in rats to low exposure in humans by the same route (Tardif et al. 1995) and to identify functional interaction thresholds for the joint toxicity of trichloroethylene and 1,1-dichloroethylene in the rat (El-Masri et al. 1996a). PBPK/PD models have been applied to further assess apparent interaction thresholds for the joint toxicity of trichloroethylene and 1,1-dichloroethylene (El-Masri et al. 1996b) and of kepone and carbon tetrachloride (El-Masri et al. 1996c) in the rat, and to extrapolate from high-dose studies of interactions of toluene and dichloromethane in animals to lower-dose exposures by a different route in humans (Pelekis and Krishnan 1997). As an example of the direct applicability to the assessment of potential hazard to human health, the study of toluene and dichloromethane illustrates the use of PBPK/PD modeling to estimate the effect of co-exposure to toluene on the induction of carboxyhemoglobinemia (adverse effect) by dichloromethane in humans at defined levels of exposure.

The above models deal with binary mixtures. Approaches to modeling simple mixtures of three or more components also are under development (Haddad and Krishnan 1998; Haddad et al. 1999a, 1999b; Krishnan and Pelekis 1995; Tardif et al. 1997). As with the models for binary mixtures, these models for three or more components are constructed by linking the models for the individual chemicals based on pairwise interaction mechanisms, and the model predictions are validated with experimental data. The reported predictions of the models may be directly useful in assessing the potential hazard of joint toxic action of the simple mixtures studied. For example, separate and linked PBPK models were used to estimate biological hazard indexes (based on blood concentrations of parent compound) for varying exposures and proportions of a three-chemical mixture (toluene, ethylbenzene, and m-xylene) (Haddad et al. 1999b). These biological hazard indexes may be relevant to the central nervous system effects of the compounds, which are considered to be due to the parent compounds.

A PBPK model for the BTEXs in the rat demonstrated the utility of this approach for predicting the blood concentrations of the parent compounds in rats following inhalation exposure to the mixture (Haddad et al. 1999a). Blood levels of the parent compounds may be relevant to central nervous system

effects. The study further demonstrates that models linked on the basis of binary interactions adequately predict the inhalation pharmacokinetics of a four-component mixture.

An approach to dealing with complex mixtures is to model portions of the mixture as a single component or "lump." This approach has been used to predict whether the metabolism of benzene to genotoxic metabolites is affected by the other components of gasoline in the mouse (Bond et al. 1998). A similar approach has been proposed and partially developed for studying the acute toxicology of JP-5, a Navy jet fuel that contains a complex mixture of petroleum hydrocarbons in the C9-C18 range (Verhaar et al. 1997; Yang et al. 1998). The focus is on the prediction of kinetics of JP-5 components in relevant tissues after acute inhalation exposure and the resultant narcosis from the dissolution of hydrocarbons in the membranes of nerve cells. The approach involves the lumping of similar mixture components into a pseudocomponent, for which necessary chemical parameters such as tissue partition coefficients are estimated. QSARs are used to estimate necessary model parameters for pseudocomponents, such as tissue-blood and air-blood partition coefficients, and metabolic rate constants.

The binary, simple, and complex mixture models discussed above are being developed and validated with acute exposure data. Results of a study using PBPK modeling and experimental data obtained at intervals during a 2-year inhalation study on dichloromethane suggest that age of the animal and continuing exposure to this chemical produce changes in disposition and metabolism, such that the use of models based on acute data may not adequately predict intermediate and chronic exposure (Thomas et al. 1996). Exposure levels in this study were relatively high (2,000 ppm, 6 hours/day, 5 days/week) and, therefore, may or may not be applicable to low exposure. To date, few of these modeling efforts include extrapolation to humans. A PBPK/PD approach for carcinogenesis is under development, but has not yet been applied to mixtures or to extrapolate to humans (Yang et al. 1998).

PBPK and PBPK/PD models are being used to efficiently design experiments to test hypotheses of interaction mechanisms and to predict whether interactions may occur at low levels of exposure, so that testing can focus on mixtures of greater concern. As this field of research progresses, however, these models are expected to become useful in more direct assessment of potential hazard to human health (Haddad and Krishnan 1998). Examples of this direct application were provided previously in this section. PBPK and PBPK/PD models could be used to explore exposure scenarios involving different intakes, proportions, and routes of exposure for the mixture components (Haddad and Krishnan 1998). In addition, such models may be used as the basis for deriving health guideline values for the mixture of concern: PBPK/PD modeling may provide estimates of an "interaction threshold" (e.g., LED_{05}, lower 95% confidence limit on an effective dose associated with a 5% extra risk) for a simple mixture that

could be used as a benchmark dose for derivation of a guidance value (Yang et al. 1998). Integration of PBPK/PD models with other approaches such as Monte Carlo simulation, response surface methodology, and QSAR is expected to further enhance predictive capability (El-Masri et al. 1997; Yang et al. 1998).

3. METHODS USED OR PROPOSED BY OTHER AGENCIES

3.1. ACGIH

ACGIH first discussed its procedure for dealing with exposure to mixtures in 1963 (ACGIH 1984); the procedure has changed but little to the present day. ACGIH (2000) recommends additivity approaches for the assessment of occupational hazard. For mixtures of two or more hazardous substances that act on the same organ system, the ratio of the exposure concentration to the threshold limit value (TLV) for each component is summed (dose addition, hazard index approach). If the sum exceeds one, then the TLV for the mixture is considered as being exceeded. Exceptions to the hazard index approach can be made when there is good reason to expect that the chief effects of the components are independent. According to ACGIH, an example would be when the components produce purely local effects on different organ systems. When the effects are expected to be independent, the TLV for the mixture is exceeded only if at least one component has a hazard quotient that exceeds unity. In effect, the hazard index for the mixture would be the highest hazard quotient for any of the components. (This resembles response addition with completely positive correlation of tolerances, Appendix A.) ACGIH recommends evaluating synergism or potentiation on a case by case basis, and further states that such interactions are characteristically exhibited at high concentrations and are less likely at low.

In the case when a process emits a number of harmful dusts, fumes, vapors, or gases, ACGIH states that frequently it may be feasible only to measure a single substance in order to evaluate the hazard. In this circumstance, the threshold limit for this substance should be reduced by a suitable factor, the magnitude of which takes into account the number, toxicity, and relative amounts of the other components typically present. This appears to be a combination indicator chemical/uncertainty factor approach. Some examples cited by ACGIH were welding, painting, and certain foundry operations.

3.2. OSHA

The Occupational Safety and Health Administration (OSHA 1993, 2001) also recommends a hazard index approach that employs the ratio of the exposure concentration to the PEL for each chemical and

sums the ratios. If the sum of the ratios exceeds one, then the exposure limit for the mixture is exceeded. OSHA does not restrict the approach to chemicals with similar effects.

3.3. NIOSH

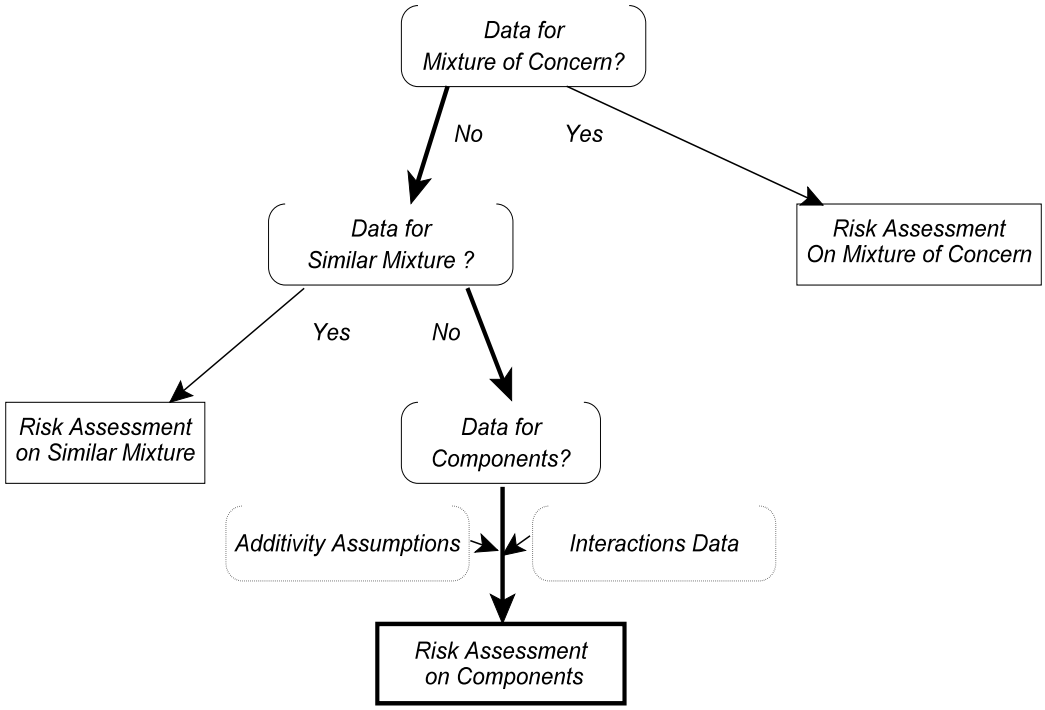
The National Institute for Occupational Safety and Health (NIOSH 1976) adopted a similar approach in recommending exposure limits for methylene chloride when carbon monoxide was also present because of the known additivity of the two chemicals with regard to formation of carboxyhemoglobin. NIOSH recommended that the sum of the ratios of each chemical to their recommended exposure limits not exceed one, and that the permissible exposure limits for methylene chloride be adjusted downward when carbon monoxide levels were greater than 9 ppm in order to keep the sum from exceeding unity. (More recent NIOSH [1992] recommendations are based on carcinogenicity.)

3.4. EPA

An overview of the EPA (1986) mixtures guidelines for risk assessment of chemical mixtures is provided in Figure 1. The guidelines recommend the use of exposure and health effects data for the mixture of concern or a similar mixture if available. If not, the use of data for the components is recommended. The components procedure is most commonly used, as indicated on the figure by the heavier arrows and box. When more than one of these approaches is feasible, EPA (1986) recommends a comparison of results from the different approaches.

The guidelines recommend the assessment of interactions data, when available, in terms of relevance to subchronic or chronic exposure and suitability for quantitatively altering the risk assessment. Interactions data are considered likely to be available mainly for pairs of chemicals, which could be assessed separately from those with no such information. The guidelines recommend, however, exploring the possibility that other components of the mixture may interfere with the interaction of the chemical pair on which quantitative interaction data are available. If interference appears likely, then quantitative alteration of the risk assessment may not be justifiable.

Figure 1. Overview of EPA Guidelines for Mixtures Risk Assessment



The assessment of the noncarcinogenic effects of the components usually proceeds by the hazard index method. Because it assumes dose additivity, the hazard index method is most suitable for chemicals with similar effects. If the mixture includes chemicals that have different effects, then EPA recommends the calculation of separate hazard indexes for each endpoint of concern. The guidelines mention that if data are sufficient to derive individual acceptable levels for a spectrum of effects, "the hazard index may suggest what types of effects might be expected from the mixture exposure." Subsequent guidance for Superfund risk assessment gave further explicit directions for the hazard index approach, including the combining of hazard indexes for multi-route exposure and the calculation of separate hazard indexes for different target organ toxicities (EPA 1989a). For carcinogenic effects, the guidelines recommend summing the risks across components, as discussed in Section 2.3.5. EPA (1999) is developing additional mixtures guidance for risk assessment, which will supplement the original EPA (1986) guidelines.

3.5. NAS/NRC

In 1972, at the request of the EPA, the NAS recommended health-based stream criteria for a large number of pollutants. A component of this appraisal was multiple chemical exposure (NAS 1974). The NAS recommended a hazard index approach, whereby the sum of the ratios of the measured concentrations to the acceptable concentrations for the components was to be kept at a level equal to or lower than unity.

In 1989, at the request of EPA, The Safe Drinking Water Committee of the National Research Council (NRC 1989) suggested possible modifications of the then current approaches for estimating the toxicity of mixtures in drinking water. The NRC suggested that mixture components be grouped by endpoint, such as specific organ toxicity and carcinogenicity in order to assess their combined risk or hazard.

For noncancer endpoints, the NRC suggested a modified hazard index that sums similar toxicities and an uncertainty factor for possible synergism, depending on the information regarding interactions and the concentrations of the components. The uncertainty factor could range from 1 to 100. If information regarding potential interactions is available and suggests interactions are not likely, or if the concentrations are low, the uncertainty factor could be set at 1. The NRC also suggested that separate hazard indexes be calculated for each toxic endpoint, including those that occur at higher exposure levels than the endpoint that is the basis for the acceptable exposure level for a component. A weighting factor would be applied to account for the lesser sensitivity of the other endpoints, unless an acceptable exposure level for the other endpoints was available. The method is similar to the TTD modification of the hazard index method, discussed previously, except the NRC further suggested summing the hazard indexes across all toxic endpoints.

For carcinogenic endpoints, the NRC concluded that it was appropriate to sum the risks (response addition with completely negative correlation of tolerances) for low-dose exposure to a mixture of carcinogens (doses with relative risks of less than 1.01).

4. ATSDR APPROACH

4.1. OVERVIEW

The ATSDR DT approach to the assessment of the joint toxic action of chemical mixtures reflects the unique nature of ATSDR's mandate to assess the public health implications associated with uncontrolled release of hazardous substances into the environment. The health effects of low-level exposures are of particular concern. As described in ATSDR's *Public Health Assessment Guidance Manual*, the determination of public health implications involves not only an assessment of potential hazard to public health based on estimated exposure levels and health guideline values, but also evaluation of uncertainties, health implications of other medical and toxicological factors and sensitive subpopulations, community-specific health outcome data, and the consideration of community health concerns (ATSDR 1992). The outcome of this process is a health assessment document that classifies the public health hazard posed by a site into one of five categories, ranging from urgent to no hazard. Follow-up activities, consistent with the degree of hazard, are recommended, and may include actions to protect public health, obtain additional health information, or obtain additional site-characterization information (ATSDR 1992; De Rosa et al. 1996; Hansen et al. 1998; Johnson and De Rosa 1995). The assessment of potential hazard to public health based on estimated exposure levels and health guideline values is called "exposure-based assessment of joint toxic action" in this Mixtures Guidance Manual, and is only one part of the overall process of evaluating the potential impact of exposure to mixtures on public health.

The strategy for exposure-based assessment of the potential impact of joint toxic action of chemical mixtures on public health is presented in detail in the text of Chapter 4, and the decision process is illustrated in flow charts. The strategy integrates the use of other ATSDR documentation, including toxicological profiles, interaction profiles, and ATSDR-sponsored research on chemical mixtures, into a screening approach for the assessment of health hazard. The conclusions from this mixtures assessment can then be taken into account along with the community-specific health outcome data, community health concerns, and biomedical judgment, to determine the public health implications and follow-up activities.

The general approach is consistent with the approach articulated by EPA (Figure 1) and used to some extent, formally or informally, by a number of agencies. This approach involves the use of exposure and toxicological information on the mixture of concern or a similar mixture as the preferred method. Exposure data are site-related. If available, toxicological information on a mixture of concern (or similar mixture) for hazardous waste sites are likely to be reviewed and evaluated in ATSDR documents,

including interactions profiles and toxicological profiles. These documents may provide MRLs or other health guideline values for the whole mixture, or guidance for other approaches. When such data are not available from ATSDR documents (or comparable documents from other agencies), an approach based on the components of the mixture is advisable, if the exposures are high enough so that the joint toxic action of the components may pose a hazard due to additivity or interactions or both. The approach will provide additional clarification of hazard, for example:

- when exposures to the components are not clearly hazardous when considered singly, but potentially hazardous due to additivity or interactions when considered together;
- when the community-specific health outcome data indicated that the site may have had an adverse impact on human health, but the exposure-based assessment of each component separately did not; or
- when the health outcome data were ambiguous or did not indicate an adverse impact on human health, but the exposure-based assessment identified a potential hazard from one or more of the components.

4.2. STEPS IN EXPOSURE-BASED ASSESSMENT OF JOINT TOXIC ACTION OF CHEMICAL MIXTURES

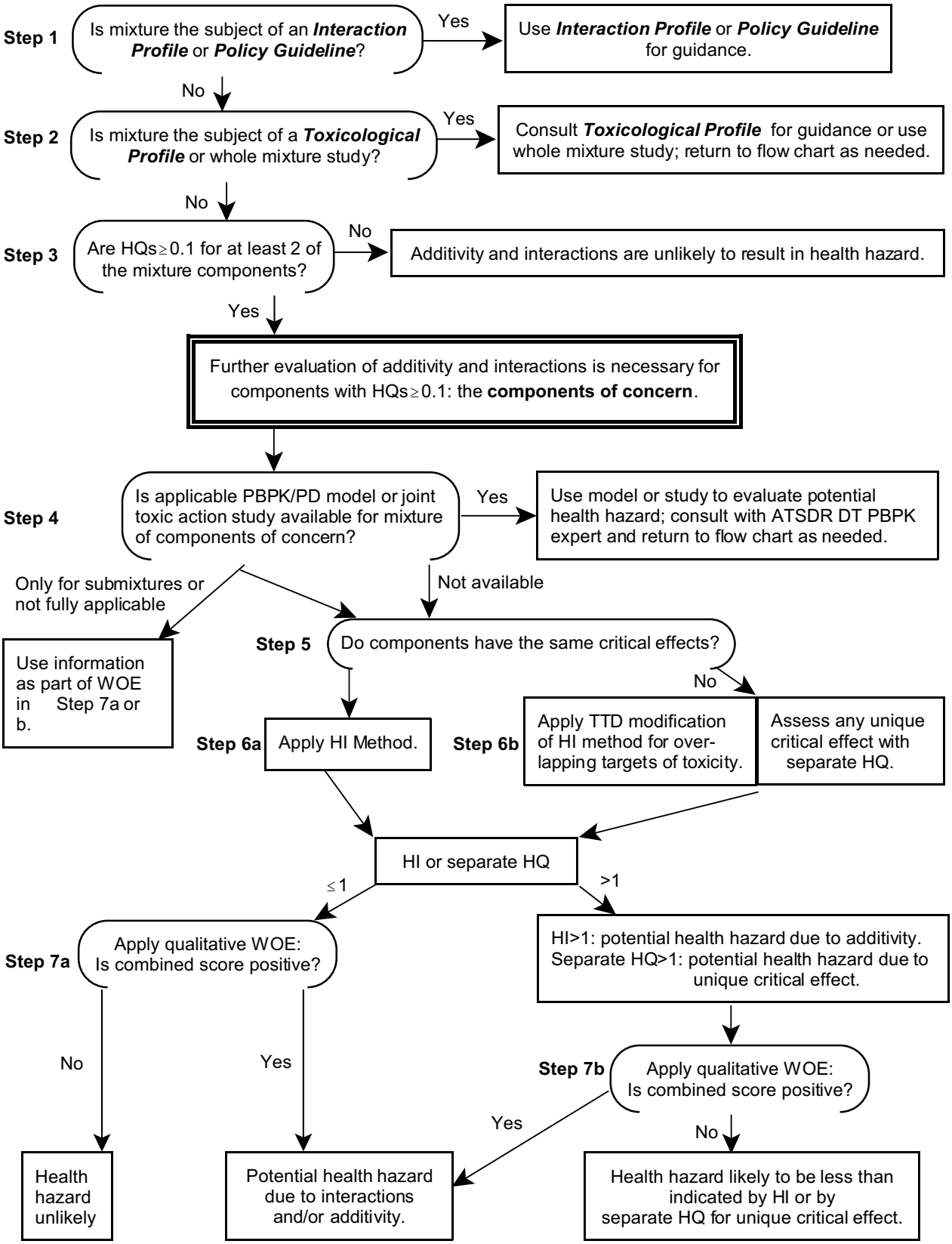
4.2.1. Procedures for Assessment of Noncarcinogenic Effects (Figure 2)

The flow chart in Figure 2 gives an overview of the steps for exposure-based assessment of the potential impact of joint toxic action on public health. The analysis of exposure pathways and intakes or concentrations should be performed using ATSDR (1992) methods for public health assessment. The process described in the flow chart and accompanying text is designed to answer the question: do the estimated levels of exposure of human populations to the mixture or to the mixture components constitute a potential health hazard? Thus, the flow chart focuses on a decision process. If a potential hazard is identified, this result does not mean that an actual public health hazard has been identified. Rather, it indicates that further evaluation using ATSDR (1992) methods for public health assessment will be needed (see Section 4.1, paragraph 1 of this mixtures guidance).

Step 1: Use Interaction Profile or Policy Guideline if available for mixture of concern.

ATSDR provides guidance on some mixtures in *Interaction Profiles* (on simple mixtures of concern for hazardous waste sites) and in *Policy Guidelines* (to date, available only for dioxins and dioxin-like

Figure 2. Strategy for Exposure-Based Assessment of Joint Toxic Action of Chemical Mixtures: Noncarcinogenic Effects *(See text for detailed explanation)*



compounds in soil [Appendix B of ATSDR 1998b]). These documents may be identified by searching the ATSDR website, and should be used for guidance. These documents recommend specific approaches to be used with waste-site-specific exposure data in order to assess potential health hazard from joint toxic action of certain mixtures. The recommended approaches may include the use of whole mixture data, assessment of components singly, PBPK/PD, TEF, hazard index, TTD, WOE, indicator chemical or other approach. The policy guideline and interaction profiles provide the needed TEF, BINWOE, and TTD values. If the document offers only partial coverage of the mixture, use as appropriate and return to flow chart for additional guidance. For example, an interaction profile may cover some of the chemicals in the mixture but not others. The flow chart can be used to further define the components of concern before deciding whether the mixture "matches" the mixture in the interaction profile, and to account for components of concern not covered by the profile. If no ATSDR documentation is available and relevant information is available from another agency, evaluate the information for suitability and use if appropriate. Otherwise, return to the flow chart at Step 2.

Step 2: Consult Toxicological Profile or use whole mixture study if available for mixture of concern.

A number of *Toxicological Profiles* deal with intentional and generated mixtures, and can be identified by searching the ATSDR website. These mixtures include fuels (e.g., ATSDR 1998a), PCBs (ATSDR 2000), CDDs (ATSDR 1998b), PAHs (ATSDR 1995b, 1999), pesticides such as toxaphene (ATSDR 1996b), and total petroleum hydrocarbons (ATSDR 1999). Some of these mixtures are assessed as whole mixtures (certain fuels and pesticides, PCBs), others are assessed with MRLs for individual components or using a fraction approach (PAHs), or on the basis of dose-additivity of the components (CDDs and CDFs; see also *Policy Guideline* in Step 1). For complex mixtures of petroleum hydrocarbons, similar components are lumped into fractions for exposure and health effects assessment, and MRLs for the fractions are recommended based on a single representative (surrogate) component or a similar mixture. For some fractions, an indicator chemical approach is used (ATSDR 1999). ATSDR has considered some mixtures, such as gasoline and Stoddard Solvent, too variable in composition for MRL derivation (ATSDR 1995a, 1995c). It was suggested, in a separate publication, that when appropriate, the most toxic (known) chemical from the mixture could be selected as a marker (indicator) chemical for the mixture, assuming that the indicator chemical would drive the risk assessment. An example is using benzene as a marker or indicator chemical for environmental exposure to automotive gasoline (Pohl et al. 1997). Alternatively, a fraction approach, as discussed previously for complex mixtures of petroleum hydrocarbons (ATSDR 1999), in conjunction with a components approach for the nonhydrocarbon components (such as methyl-*t*-butyl ether), may be useful for gasoline. If the toxicological profile does not provide MRLs or recommendations for health assessment approaches, and relevant documentation

from other agencies is not available or is not suitable, the literature can be searched for studies on the mixture of concern (whole mixture), and any available studies can be evaluated for possible use as the basis for a MRL, or to identify potential health effects of concern from exposure to the mixture. Studies of wildlife or companion animals exposed to site-related chemicals may be useful in identifying that a hazard exists at environmental levels of exposure, if evaluated for relevance to potential effects on human health. MRLs are derived in accordance with ATSDR (1996a) guidance. Additional guidance regarding implementation of a "whole mixture" approach is provided in ATSDR (2001). If information sufficient to conduct a mixtures assessment is not identified, return to the flow chart.

Step 3: If no ATSDR document is available for the mixture of concern, select components of concern.

If Steps 1 and 2 do not reveal suitable approaches or information for a mixtures assessment, or if the information is incomplete, a components approach is employed. The components approach focuses on components that are likely contributors to health hazard either because their individual exposure levels exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard.

Components for which exposures are less than a ratio of 0.1 relative to noncancer health guidelines (i.e., have hazard quotients less than 0.1, HQs<0.1) are considered unlikely to pose a health hazard due to interactions, and unless there are a relatively large number of components that act similarly, are not likely to pose an increased hazard due to additivity. These components are eliminated from further consideration in Step 3. The value 0.1 is chosen as a reasonable point of departure for simple mixtures consisting of approximately 10 components or fewer. If *all* of the components have HQs<0.1, additivity and interactions among the components are unlikely to result in a hazard to public health, and further assessment of the mixture is not necessary. (If only one component is present at a HQ≥0.1, and if the HQ for that component exceeds unity, this situation is not considered a mixtures problem. The single component should be evaluated further using ATSDR [1992] public health assessment guidance.)

If two or more components have HQs≥0.1, these chemicals are components of concern for joint toxic action. Proceed with the evaluation of additivity and interactions in Steps 4-7 for these components of concern. Judgment should be used, however, in applying this value. With a mixture of more than 10 components that act similarly, or with several components with HQs just slightly below 0.1 and other HQs above 0.1, a slightly lower point of departure may be appropriate (see Section 4.2.2.7 for an example).

When used in the assessment of hazardous waste sites, the hazard quotient is commonly reported to one significant figure (EPA 1989a). For example, a hazard quotient of 0.13 is rounded to 0.1, and a hazard quotient of 1.6 is rounded to 2.

Step 4: Evaluate and use PBPK/PD model or joint toxic action studies, if available and appropriate.

If a PBPK, or PBPK/PD model and/or joint toxic action study is available for the complete mixture of components of concern, evaluate its relevance to human exposure by the anticipated route(s) and duration, and to the noncancer health effects of concern for the components. Studies of joint toxicokinetics or joint toxic action are commonly performed to validate the models. The effects of concern will include the critical effects and any relatively sensitive effects in common among two or more of the mixture components. The critical effect is the effect that is the basis for the MRL (or RfD or RfC). Examples of existing PBPK and PBPK/PD models and their potential usefulness were presented in Section 2.3.7 of this guidance.

Evaluation of the model also should include whether the models for the individual components have been linked in a reasonable manner, based on the components' toxicokinetics and mechanisms of action, and the extent of validation of the model. If a model appears directly useful for predicting the potential health hazard of defined levels of exposure to the components of concern, consult with an ATSDR DT PBPK/PD expert regarding the possibility of obtaining and using the model. The literature reports of some models or studies of joint action may be directly useful, for example, if they report apparent threshold exposures for interactions relevant to human exposure or that the components will not interact. This information can be used in Step 7 during the WOE evaluation and as part of the rationale for the components approach. The availability of linked PBPK and PBPK/PD models for mixtures is limited as of this writing, but research in this area is highly active. Therefore, update searching of an appropriate database such as TOXLINE should be conducted to identify pertinent PBPK or PBPK/PD models. For some mixtures, models may be available only for submixtures, including pairs of components, within the mixture. In this case, the hazard index method (Step 6a) or the TTD modification of the hazard index or separate HQ (Step 6b) can be chosen as appropriate, and reported results of the modeling for pairs of components can be used as part of a WOE approach. If no suitable models are available or if the models are to be used as part of the WOE evaluation, proceed to Step 5.

Step 5: Evaluate whether components have the same or different critical effects.

Assess whether the components that contribute to a particular exposure pathway of concern appear to affect primarily the same endpoints, particularly in terms of critical effects or critical target organs. If so, apply the hazard index method (Step 6a). If the components appear to have a variety of critical effects, apply the TTD modification to the hazard index (Step 6b). If most, but not all, of the mixture's components have the same critical effect, the hazard index method can be chosen on the grounds of practicality.

Step 6a: Apply hazard index method to components with similar critical effects.

The hazard index method was discussed in Section 2.3.1. A hazard index is estimated for a specific receptor population, for the duration and pathway of concern. The exposure units should be the same as the units for the health guideline (e.g., mg/kg/day for oral exposure pathways, when using oral MRLs [or RfDs] as health guideline values, and units of air concentration for inhalation exposure pathways, when using inhalation MRLs [or RfCs] as health guideline values). For example, adapting equations 2 and 1(b) for use with MRLs (and RfDs for components lacking oral MRLs) results in the following equation for the oral hazard index (HI_{oral}) for pathways involving oral exposure:

$$HI_{oral} = \sum_{i=1}^{n} HQ_{i\ oral} = \sum_{i=1}^{n} \frac{E_{i\ oral}}{MRL_{i\ oral}\ or\ RfD_i} \qquad (7)$$

where $HQ_{i\ oral}$ is the oral hazard quotient, $E_{i\ \mathrm{oral}}$ is the oral exposure in mg/kg/day, and $MRL_{i\ oral}\ or\ RfD_i$ is the oral MRL or RfD in units of mg/kg/day, for the i^{th} component.

If the resulting hazard index exceeds one, the mixture constitutes a *potential* health hazard due to additivity. Further evaluation of interactions is needed to gauge the extent of the hazard (Step 7). If the resulting hazard index is less than or equal to one, further evaluation of interactions is required to assess the potential for interactions to increase the apparent hazard (Step 7). As was the case for the hazard quotient (Step 3), the hazard index is rounded to one significant figure (EPA 1989a).

Step 6b: Apply TTD modification of hazard index method for components with different critical effects.

The TTD modification to the hazard index method was discussed in Section 2.3.2, and example equations were presented there. Separate hazard indexes are estimated for each major endpoint or target organ affected by two or more components of the mixture (i.e., the overlapping targets of toxicity). The MRL (or RfD or RfC) for a component is used when the hazard index is for the endpoint on which that health guideline is based. TTDs are used for the other major effects of the component. The equations

are similar to equation 7 above. For example, a hazard index for hepatic effects from a pathway involving oral exposure is calculated as follows:

$$HI_{oral\ hepatic} = \sum_{i=1}^{n} HQ_{i\ oral\ hepatic} = \sum_{i=1}^{n} \frac{E_{i\ oral}}{MRL_{i\ oral}\ or\ RfD_i\ or\ TTD_{i\ oral\ hepatic}} \quad \textbf{(8)}$$

where $HI_{oral\ hepatic}$ is the oral hazard index for hepatic effects, HQ_i is the hazard quotient, $E_{i\ \mathrm{oral}}$ is the oral exposure in mg/kg/day, and $MRL_{i\ oral}\ or\ RfD_i\ or\ TTD_{i\ oral\ hepatic}$ is the oral MRL or RfD or TTD for hepatic effects in units of mg/kg/day, for the i^{th} component.

If any of the endpoint-specific hazard indexes exceed one, the mixture constitutes a *potential* health hazard due to additivity. Further evaluation of interactions is needed to gauge the extent of the hazard (Step 7). If all the endpoint-specific hazard indexes are one or less than one, further evaluation is required to assess the potential for interactions to increase the hazard (Step 7). In addition, if any component of the mixture has a unique critical effect (effect not produced by any of the other components), this effect should be addressed by assessing whether the hazard quotient exceeds unity, in which case it would be considered a *potential* health hazard. The qualitative WOE method also should be applied (Step 7) to gauge whether any of the other mixture components may influence the toxicity of this component with regard to this critical effect.

Step 7(a and b): Apply Qualitative WOE.

The qualitative WOE methodology, summarized previously in Section 2.3.3, provides a means of predicting joint toxic action when the data are not sufficient (as is usually the case) to use more quantitative means. The BINWOE determinations are used to make judgments regarding whether the health hazard may be greater or lesser than would be predicted on the basis of the hazard index alone. BINWOEs need to be route-, duration-, and endpoint- or target-organ-specific. This specificity may be accommodated within a single BINWOE determination, or through separate BINWOE determinations. Before using a BINWOE, make sure it is applicable to the route(s), duration(s), and effect(s) of concern for the particular assessment.

The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2\text{-}N)/2$. Thus, this mixture of 4 components has 6 pairs of components and potentially 11 BINWOEs for a given route, duration, and effect. Suppose nine of the BINWOEs are greater-than-additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three

BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the weight of evidence suggests that the mixture is likely to pose a greater hazard than that indicated by the hazard index.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater than additive, some less than additive, and some additive BINWOEs). In this case, still using the qualitative WOE approach, the qualitative BINWOE scores are converted to numerical scores, and the scores are summed to give a combined score. If the combined BINWOE score is positive and significantly different from zero, the weight of evidence suggests that the mixture is likely to pose a greater hazard than indicated by the hazard index. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the weight of evidence suggests that the health hazard is likely to be less than indicated by the hazard index. If the combined BINWOE score is zero or close to zero, the weight of evidence does not suggest that interactions will alter the potential health hazard as represented by the hazard index. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index.

Step 7a: This part of Step 7 describes the application of the qualitative WOE to hazard indexes that are less than or equal to unity ($HI \leq 1$). If the BINWOE alphanumeric scores indicate greater than additivity, or if the combined BINWOE numerical score is positive and significantly greater than zero, and particularly if the hazard index is near unity, these levels of exposure to the mixture constitute a *potential* health hazard. Further evaluation using the methods in ATSDR (1992) is necessary. Conversely, if the BINWOE alphanumeric scores indicate less than additivity or additivity, or the combined numerical score is negative or very close to zero, the mixture is unlikely to be a health hazard at the hazardous-waste-site related exposure levels.

Step 7b: This part of Step 7 describes the application of the qualitative WOE to hazard indexes that are greater than unity ($HI > 1$). If the BINWOE alphanumeric scores indicate greater than additivity or additivity, or if the combined BINWOE numerical score is positive, these levels of exposure to the mixture constitute a *potential* health hazard due to interactions and/or additivity. Further evaluation using the methods in ATSDR (1992) is necessary. Conversely, if the BINWOE alphanumeric scores indicate less than additivity, or the combined numerical score is negative and significantly different from zero, the mixture health hazard is likely to be less than indicated by the hazard index. Further evaluation using the methods in ATSDR (1992) is needed.

4.2.2. Example Applications of Exposure-Based Assessment of Joint Toxic Action for Noncarcinogenic Effects of Chemical Mixtures

The following examples are hypothetical examples chosen to illustrate how the procedures outlined in Figure 2 can be applied to a variety of exposure situations. Each example is for a single pathway and duration (assume intermediate or chronic) of exposure.

4.2.2.1. Residential Soil Contamination with CDDs and CDFs

Under Step 1, the ATSDR website is searched for relevant information, and the draft policy guideline for dioxin and dioxin-like compounds in soil (De Rosa et al. 1997a) is identified and downloaded. Further investigation locates the final policy guideline published as an appendix to the CDDs profile (ATSDR 1998b). This policy guideline provides the necessary guidance for health effects assessment of these mixture components; the guideline applies to noncarcinogenic effects (and to carcinogenic effects). Additional background can be obtained from the supporting documentation (De Rosa et al. 1997b, 1997c) and the toxicological profiles on CDDs and CDFs (ATSDR 1994, 1998b).

4.2.2.2. Groundwater Contamination with Chemicals A, B, and C

An interaction profile is available on this particular common mixture and can be identified by searching the ATSDR website. The interaction profile provides specific guidance on an approach for the assessment of joint toxic action for noncarcinogenic (and carcinogenic) effects of this mixture. Use the recommended approach to conduct exposure-based assessment of joint toxic action to screen for potential health hazard of the mixture, and use the ATSDR (1992) guidance for public health assessment for the other aspects of public health assessment.

4.2.2.3. Residential Soil Contamination with Toxaphene

Although no policy guideline or interaction profile (Step 1) is available for this mixture, a toxicological profile is available (Step 2), and provides MRLs for noncarcinogenic effects (and risk-specific doses, slope factor, and unit risk for carcinogenic effects) of toxaphene assessed as a whole mixture. These health guideline values and other information in the profile are used in accordance with ATSDR guidance for public health assessment (ATSDR 1992).

4.2.2.4. Groundwater Contamination with Chemicals D, E, F, and G

No policy guideline, interaction profile, or toxicological profile is identified for this mixture (Steps 1 and 2, Figure 2), but toxicological profiles are available for the individual chemicals. A components approach is therefore initiated. The following four "cases" are hypothetical and are presented to illustrate the use of the approach in Figure 2 for mixtures with a relatively small number of components.

Case 1: The hazard quotient (ratio of the exposure dose [oral intake in mg/kg/day] to the oral MRL [or RfD if MRL not available]) for each chemical is estimated as follows.

chemical D:	(exposure dose)/MRL	= (0.05 mg/kg/day)/(0.5 mg/kg/day)	= 0.1
chemical E:		= (0.03 mg/kg/day)/(0.09 mg/kg/day)	= 0.3
chemical F:		= (2 mg/kg/day)/(0.5 mg/kg/day)	= 4
chemical G:		= (12 mg/kg/day)/(2 mg/kg/day)	= 6

Thus, the hazard quotients for chemicals D, E, F, and G are 0.1, 0.3, 4, and 6, respectively. Because the hazard quotients for chemicals F and G are above unity, these individual components can be considered potential health hazards. For all four chemicals, the hazard quotients are at least 0.1 (HQs $\geq$ 1), and all four are selected as components of concern (Step 3). Further evaluation is necessary to assess the potential impact of additivity and interactions on the degree of hazard. No PBPK/PD or PBPK model is available for the mixture (Step 4). The critical effects for all four components are the same, hepatic (Step 5). Therefore, a hazard index is calculated as the sum of the hazard quotients (HI = 0.1 + 0.3 + 4 + 6 = 10.4, rounded to 10) (Step 6a). The magnitude of the hazard index indicates a potential health hazard due to additivity. Evaluation of interactions (Step 7b for HI>1) is needed. BINWOE scores that are relevant to the route, duration, and endpoint for the six chemical pairs are provided by ATSDR. The BINWOEs are additive for the effect of chemical D on the toxicity of chemical E, less than additive for E on D, and indeterminate for F on D. The remaining nine BINWOEs are greater than additive. The additive and indeterminate BINWOEs are for effects on the toxicities of components with relatively low HQs (D and E), whereas the greater-than-additive BINWOEs include effects of the components with relatively high HQs (F and G) on each other's toxicity, and also reflect relatively high confidence (high numerical BINWOE scores). Summing the BINWOE scores results in a combined score of +4.99. These results indicate that the hazard is likely to be greater than would be predicted on the basis of the default assumption of additivity (the hazard index of 10). Thus, the mixture is a *potential* health hazard at the estimated levels of exposure, and will be subjected to further evaluation according to procedures in ATSDR's guidance for public health assessment (ATSDR 1992).

Case 2a: The hazard quotients for chemicals D, E, F, and G are estimated at 0.02, 0.2, 0.5, and 0.4 in a manner similar to that shown for Case 1. Three of the chemicals (E, F, and G) have HQs $\geq$ 0.1; these chemicals are components of concern as indicated by Step 3. The hazard quotient of 0.01 for chemical D, however, is an order of magnitude lower than the other three, and much lower than 0.1. Chemical D is considered unlikely to have an impact due to additivity or interactions, so it is dropped from further consideration. No PBPK/PD or PBPK model is available for the three-component mixture, but a PBPK model is available for a binary mixture of chemicals E and F, and is applicable to oral exposure (Step 4). The model will be considered subsequently during the evaluation of interactions for this pair. The critical effects (Step 5) for the three components are hepatic effects. The hazard index (Step 6a) is 1 (HI = 0.2 + 0.5 + 0.4 = 1.1, rounded to 1). To further assess the potential hazard, a qualitative WOE evaluation is undertaken (Step 7a for HI $\leq$ 1), using relevant BINWOEs available from ATSDR. The BINWOEs for the mixture of chemicals E and F were based in part on the PBPK model predictions. Four of the six BINWOEs for the three possible pairs are greater than additive (positive) and two are additive (0); thus, the weight of evidence suggests the hazard will be greater than indicated by the hazard index. The combined BINWOE score will be positive. Consistent with Step 7, it is concluded that the mixture constitutes a *potential* health hazard at the estimated exposure levels. It should be evaluated further using ATSDR guidance for public health assessment (ATSDR 1992).

Case 2b: Identical to Case 2a, except that, in step 7, the six BINWOEs for the three possible pairs are less than additive (negative) and additive. Because the hazard index is 1, and the BINWOEs suggest that the hazard will be less than predicted by the hazard index, it is unlikely that the mixture would be a health hazard at the estimated exposure levels.

Case 3: The hazard quotients for chemicals D, E, F, and G are 0.8, 1, 2, and 0.8. As indicated by Step 3, further evaluation is necessary. No PBPK/PD or PBPK model is available for the mixture (Step 4). The components all have the same critical effects (hepatic) (Step 5). The hazard index (Step 6a) is 5 (HI = 0.8 + 1 + 2 + 0.8 = 4.6, rounded to 5). The mixture is considered to constitute a potential health hazard on the basis of additivity. Five of the BINWOEs are less than additive, including some scores for the effects on the toxicity of chemical F, which has the highest hazard quotient. The remaining seven BINWOEs are additive. Thus, the qualitative WOE approach (Step 7b) would indicate that the hazard may be less than indicated by the hazard index. Although this result indicates that the health hazard is likely to be less than indicated by the hazard index of 5, the result should be interpreted with care. Exposure to a mixture of hazardous substances that may act antagonistically may be considered to be less hazardous than if the joint toxic action of those substances were additive or synergistic, but it does not

rule out all concern, particularly when the hazard index is not close to 1. Further evaluation using ATSDR (1992) guidance for public health assessment is needed.

4.2.2.5. Groundwater Contamination with Chemicals H, I, J, K, and L

No suitable documents for the mixture are available (Steps 1 and 2). The hazard quotients for chemicals H, I, J, K, and L are 0.1, 0.2, 0.5, 0.4, and 0.3; all four chemicals are components of concern (Step 3). PBPK/PD or PBPK models are not available (Step 4). The components have different critical effects (hepatic for chemicals H and I, renal for chemical J, and hematological for chemical K, and dermal for chemical L) (Step 5). Estimation of endpoint-specific hazard indexes using the TTD modification of the hazard index (Step 6b), and TTDs available from ATSDR, results in an hazard index of 0.6 for hepatic effects, 0.7 for renal effects, 0.5 for hematological effects, and 0.4 for developmental effects (included because three of the components have developmental effects). Dermal effects are a "unique critical effect" in that they are the critical effect of one chemical (L), but are not caused by any of the other chemicals; the hazard quotient for this effect is 0.3 as noted above. Thus, the endpoint-specific hazard indexes all are less than one and the hazard quotient for the unique critical effect is also less than one. A qualitative WOE evaluation is undertaken (Step 7a). BINWOE evaluations are available for hepatic (less than additive and additive), renal (less than additive), and hematological effects (less than additive or additive). BINWOEs for the effects of the other mixture components on the dermal toxicity of chemical L are less than additive or indeterminate. Little information on interactions or mechanisms specifically relevant to developmental effects is available, so all evaluations for developmental are indeterminate or additive with low scores. Concern for greater-than-additive interactions for developmental toxicity is low, however, because greater-than-additive interactions are not seen for the four other endpoints. Because the endpoint-specific hazard indexes are less than one, the hazard quotient for the unique critical effect is less than one, and the WOE evaluations are mainly additive to less than additive (with none greater than additive), it is concluded that the mixture is not likely to be a health hazard at the estimated levels of exposure.

4.2.2.6. Air Contamination with Chemicals M, N, and O

No interaction profile or guidance policy is available for this mixture (Step 1), and toxicological profiles and MRLs are not available for the mixture (Step 2), but are available for the individual components. The hazard quotients (ratios of exposure concentrations to inhalation MRLs, or RfCs if MRLs not

available) for the components are 0.3, 0.4, and 0.3:

chemical M:	(Exposure concentration)/MRL	= (0.2 ppm)/(0.6 ppm)	= 0.3
chemical N:		= (0.08 ppm)/0.2 ppm)	= 0.4
chemical O:		= (0.6 ppm)/(2 ppm)	= 0.3

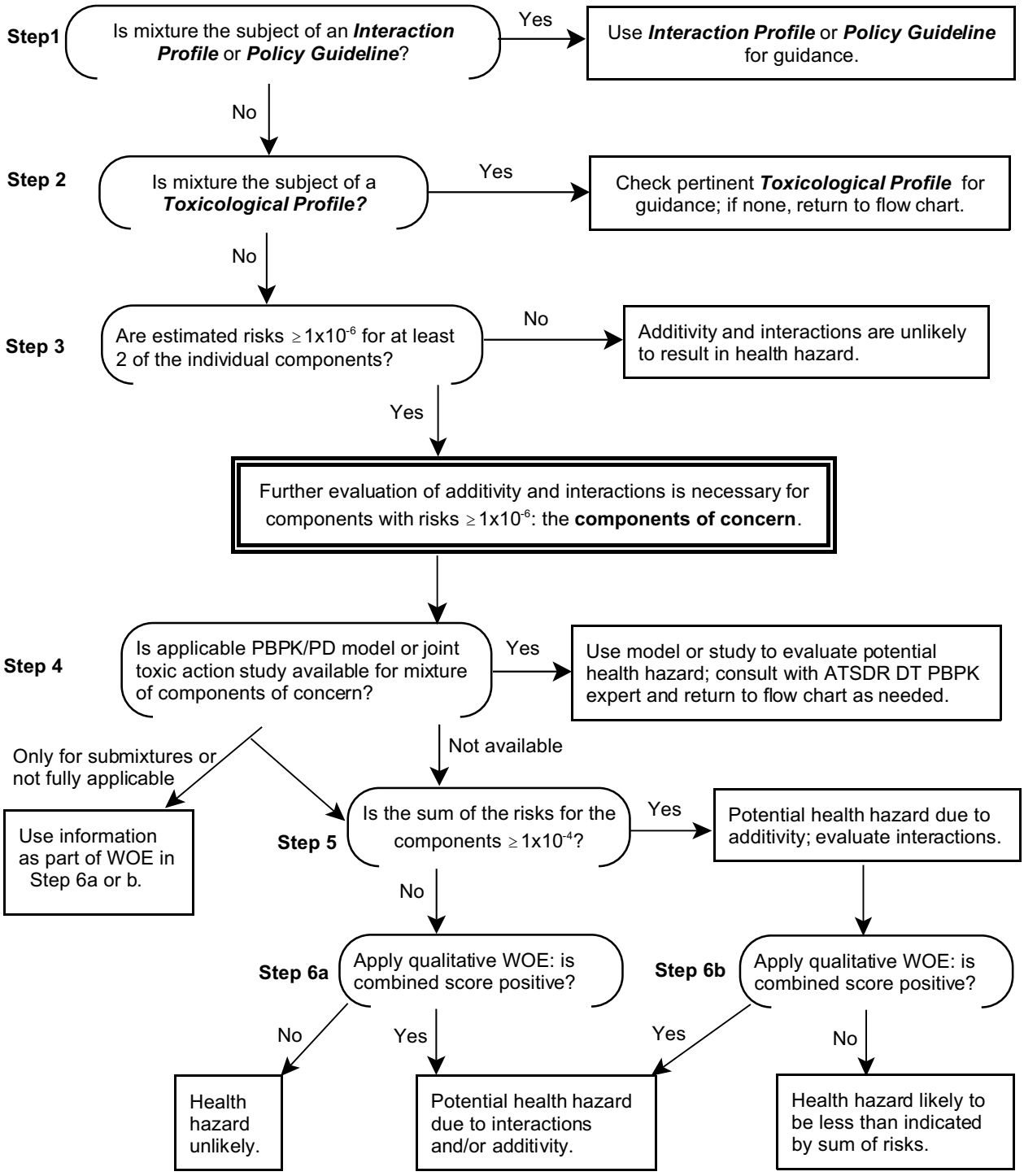
Thus, all three components are components of concern (Step 3). PBPK/PD models are available for all three possible pairs (M and N, M and O, N and O) but not for the entire three-component mixture (Step 4). The components have the same critical effect, renal (Step 5). The hazard index (Step 6a) is 1 (HI = 0.3 + 0.4 + 0.3). Based on the PBPK/PD models, which extrapolate from animal data to humans by the inhalation route and have been further calibrated with human inhalation data, the site-specific exposure levels for each pair of chemicals are within the exposure range where dose-additivity is predicted by the model. Less-than-additive results are predicted at higher exposure levels. The models were published recently and therefore were not cited in the BINWOEs available from ATSDR, but conclusions are reasonably consistent with the BINWOEs. Therefore, the mixture is considered unlikely to constitute a health hazard at the estimated exposure levels.

4.2.2.7. Groundwater Contamination with 12 Chemicals

No interaction profile or guidance policy is available for this mixture, but toxicological profiles and MRLs or other comparison values are available for the components. Hazard quotients range from 0.0009 to 0.3, with only one component having a hazard quotient of 0.1 or more. Although the usual conclusion, according to Step 3, would be that the mixture is unlikely to pose a health hazard due to additivity or interactions of the components, in this case, because of the larger number of components, components slightly below the point of departure (0.1) for the hazard quotient are evaluated further.

Five of the components have hazard quotients that are 0.01 or less, well below the point of departure, and therefore are dropped from the assessment. Six components have hazard quotients approaching 0.1 (i.e., 0.07, 0.08, 0.07, 0.09, 0.09, and 0.08), and are retained, along with the component with the hazard quotient of 0.3, for further assessment. PBPK or PBPK/PD models are not available for the full mixture or for any of the pairs of chemicals within the mixture (Step 4). Six of the seven components of concern are organic compounds that affect the liver and nervous system. The critical effects are hepatic for four of the organics and neurological for two, but chemical-specific LOAELs for these two endpoints vary by less than a factor of two, as do the NOAELs. The seventh component is an inorganic chemical, for which the critical effect is renal; this component also affects the liver at higher exposures. The TTD approach

Figure 3. Strategy for Exposure-Based Assessment of Joint Toxic Action of Chemical Mixtures: Carcinogenic Effects *(See text for detailed explanation)*



would be preferable for this mixture of concern, but because of the number of components and the similarity of effects for six of the seven, the hazard index approach (Step 6a) could be chosen as a more practical interim approach. The hazard index for the six organic components with similar effects is 0.7 (rounded from 0.69). Inclusion of the hazard quotient of 0.09 for the component with renal effects would result in a hazard index of 0.8 (rounded from 0.78).

The potential impact of interactions should be evaluated. For this mixture, if the 6 components with similar endpoints are evaluated with WOE method, there would be 15 pairs of chemicals, requiring 30 BINWOEs. If BINWOEs are available from ATSDR for these pairs, then further evaluation as described in Step 7 can be readily undertaken. If the TTD approach is chosen, TTDs will be needed for the endpoints mentioned above, and possibly for others, and may be available from ATSDR. BINWOEs will also be needed for these endpoints. If BINWOEs or TTDs are not readily available, biomedical judgment and careful consideration of the community-specific health outcome data and community health concerns could be used to decide whether further analysis is needed, using ATSDR guidance for public health assessment (ATSDR 1992). If the decision is to pursue further analysis, ATSDR DT mixtures toxicologists should be consulted, and methods outlined in Section 2.3.2 (TTDs) and Section 2.3.3 (WOE) can be used. Additional detail regarding the derivation of BINWOEs is provided in ATSDR (2001).

4.2.3. Procedures for Assessment of Carcinogenic Effects (Figure 3)

The flow chart in Figure 3 gives an overview of the steps for exposure-based assessment of the potential impact of joint toxic action on public health. The analysis of exposure pathways and intakes or concentrations should be performed using ATSDR (1992) methods for public health assessment. The process described in the flow chart and accompanying text is designed to answer the question: do the estimated levels of exposure of human populations to the mixture or to the mixture components constitute a potential health hazard? Thus, the flow chart focuses on a decision process. If a potential hazard is identified, this result does not mean that an actual public health hazard has been identified. Rather, it indicates that further evaluation using ATSDR (1992) methods for public health assessment will be needed (see Section 4.1, paragraph 1 of this mixtures guidance).

Step 1: Use Interaction Profile or Policy Guideline if available for mixture of concern.

ATSDR provides guidance on some mixtures in *Interaction Profiles* (on simple mixtures of concern for hazardous waste sites) and in *Policy Guidelines* (to date, available only for dioxins and dioxin-like compounds in soil [ATSDR 1998b, Appendix B]). These documents may be identified by searching the ATSDR website, and should be used for guidance. These documents recommend specific approaches to be used with waste-site-specific exposure data in order to assess potential health hazard from joint toxic action of certain mixtures. The recommended approaches may include the use of whole mixture data, assessment of components singly, PBPK/PD, TEF, WOE, indicator chemical or other approach, and provide the needed TEFs and BINWOEs. If the document offers only partial coverage of the mixture, use as appropriate and return to flow chart for additional guidance. For example, an interaction profile may cover some of the chemicals in the mixture but not others. The flow chart can be used to further define the components of concern before deciding whether the mixture "matches" the mixture in the interaction profile, and to account for components of concern not covered by the profile. If no ATSDR documentation is available and relevant information is available from another agency, evaluate the information for suitability and use if appropriate. Otherwise, return to the flow chart.

Step 2: Consult Toxicological Profile if available for mixture of concern.

In addition, a number of *Toxicological Profiles* deal with intentional and generated mixtures, and can be identified by searching the ATSDR website. These mixtures include fuels (e.g., ATSDR 1998a), PCBs (ATSDR 2000), CDDs (ATSDR 1998b), PAHs (ATSDR 1995b, 1999), pesticides such as toxaphene (ATSDR 1996b), and total petroleum hydrocarbons (ATSDR 1999). Some of these mixtures are assessed as whole mixtures (certain pesticides, PCBs), others can be assessed using a relative potency approach for carcinogenicity (PAHs), or on the basis of dose-additivity of the components (CDDs and CDFs; see also *Policy Guideline* in Step 1). ATSDR provides perspective on the relevance to public health of the carcinogenicity data, reports the conclusions of other agencies that assess carcinogenicity, and reports EPA dose-response assessment values (e.g., slope factors, unit risks). IRIS may also be consulted for these values. If the toxicological profile (or IRIS or other suitable documentation from other agencies) does not provide recommendations for health assessment approaches, return to the procedures in the flow chart.

Step 3: If no ATSDR document is available for the mixture of concern, select components of concern.

As was the case for noncarcinogenic effects, the following approach for carcinogenic effects focuses on components that are likely contributors to health hazard either because their individual exposure levels exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard. Thus, components for which exposures do not exceed guideline values (based on an increased lifetime cancer risk of $1x10^{-6}$, a conservative level) are considered unlikely to pose increased risk due to interactions or additivity, and are dropped from further consideration in Step 3. If all the components have risks less than $1x10^{-6}$, additivity and interactions among the components are unlikely to result in a hazard to public health, and further assessment of the mixture is not necessary. (If only one component is present at a risk$\geq 1x10^{-6}$, and if the risk for that component is $\geq 1x10^{-4}$, this situation is not considered a mixtures problem. The single component should be evaluated further using ATSDR [1992] public health assessment guidance.)

If estimated risks equal or exceed $1x10^{-6}$ for two or more of the components, these chemicals are components of concern for joint toxic action. Proceed with the evaluation of additivity and interactions in Steps 4-6 for these components of concern.

Increased lifetime cancer risks are estimated by multiplying the slope factor (for oral exposure) or unit risk (for inhalation exposure) by the estimated exposure in the same units (mg/kg/day for oral, air concentration for inhalation). When used in the assessment of hazardous waste sites, risks are commonly reported to one significant figure (e.g., an estimated risk of $1.4x10^{-5}$ is rounded to $1x10^{-5}$, and $9.8x10^{-7}$ is rounded to $1x10^{-6}$).

Step 4: Evaluate and use PBPK/PD model or joint toxic action studies, if available and appropriate.

If a PBPK or PBPK/PD model is available for the mixture of components of concern, evaluate its relevance to human exposure by the anticipated route(s) and duration, and to the cancer health effects of the components. Studies of joint toxicokinetics or joint toxic action are commonly performed to validate the models. Examples of existing PBPK models and their potential usefulness were presented in Section 2.3.7 of this guidance.

Evaluation of the model also should include whether the models for the individual components have been linked in a reasonable manner, based on the components' toxicokinetics and mechanisms of action, and the extent of validation of the model. If a model appears directly useful for predicting the potential

health hazard of defined levels of exposure to the components of concern, consult with an ATSDR DT PBPK/PD expert regarding the possibility of obtaining and using the model. The literature reports of some models or studies of joint toxic action may be directly useful, for example, if they report apparent threshold exposures for interactions relevant to human exposure or that the components will not interact. This information can be used in Step 6 during the WOE evaluation. The availability of linked PBPK and PBPK/PD models for mixtures is limited as of this writing, but research in this area is highly active. Therefore, update searching of an appropriate database such as TOXLINE should be conducted to identify pertinent PBPK or PBPK/PD models. For some mixtures, models may be available only for submixtures, including pairs of components, within the mixture. In this case, the reported results of the modeling for pairs of components can be used as part of a WOE approach. If no suitable models are available or if the models are to be used as part of the WOE evaluation, proceed to Step 5.

Step 5: Sum the cancer risks.

If the sum of the cancer risks for a pathway exceeds the point of departure for significant impact on lifetime cancer risk, the mixture constitutes a *potential* health hazard due to additivity. A risk of $1x10^{-4}$ (1 in 10,000) is selected as the point of departure for significant risk (ATSDR 1992; De Rosa et al. 1993). Further evaluation of interactions, using the methods described in Step 6, is needed to gauge the extent of the hazard. If the sum of the cancer risks is less than the point of departure, further evaluation of interactions is required to assess the potential for interactions to increase the apparent hazard (Step 6).

Step 6: Apply qualitative WOE.

The qualitative WOE methodology, summarized previously in Section 2.3.3, provides a means of predicting joint toxic action when the data are not sufficient (as is usually the case) to use more quantitative means. The BINWOE determinations are used to make judgments regarding whether the health hazard may be greater or lesser than would be predicted on the basis of the sum of the cancer risks alone. BINWOES need to be route-, duration-, and endpoint-specific. This specificity may be accommodated within a single BINWOE determination, or through separate BINWOE determinations. Before using a BINWOE, make sure it is applicable to the route(s), duration(s), and effect(s) of concern for the particular assessment.

The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2-N)/2$.

Thus, this mixture of 4 components has 6 pairs of components and potentially 11 BINWOEs for carcinogenicity by a given route and duration. Suppose nine of the BINWOEs are greater-than-additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the weight of evidence suggests that the mixture is likely to pose a greater hazard than that indicated by the sum of the risks.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater than additive, some less than additive, and some additive BINWOEs). In this case, still using the qualitative WOE approach, the qualitative BINWOE scores are converted to numerical scores, and the scores are summed to give a combined score. If the combined BINWOE score is positive and significantly different from zero, the weight of evidence suggests that the mixture is likely to pose a greater hazard than indicated by the sum of the risks. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the weight of evidence suggests that the health hazard is likely to be less than indicated by the sum of the risks. Professional judgment is used in the interpretation of the impact of the WOE on the sum of the estimated cancer risks.

Step 6a: This portion of Step 6 describes the application of the qualitative WOE when the sum of the risks for the components is less than $1x10^{-4}$. If the BINWOE alphanumeric scores indicate greater than additivity, or if the combined BINWOE numerical score is positive and significantly greater than zero, and particularly if the sum of the risks is near $1x10^{-4}$, these levels of exposure to the mixture constitute a *potential* health hazard. Further evaluation using the methods in ATSDR (1992) is necessary. Conversely, if the BINWOE alphanumeric scores indicate less than additivity or additivity, or the combined numerical score is negative or very close to zero, the mixture is unlikely to be a health hazard at the hazardous-waste-site related exposure levels.

Step 6b: This portion of Step 6 describes the application of the qualitative WOE when the sum of the risks is greater than or equal to $1x10^{-4}$. If the BINWOE alphanumeric scores indicate greater than additivity or additivity, or if the combined BINWOE numerical score is positive, these levels of exposure to the mixture constitute a *potential* health hazard due to interactions and/or additivity. Further evaluation using the methods in ATSDR (1992) is necessary. Conversely, if the BINWOE alphanumeric scores indicate less than additivity, or the combined numerical score is negative and significantly less than zero, the mixture health hazard is likely to be less than indicated by the sum of the risks. Further evaluation using the methods in ATSDR (1992) is needed.

4.2.4. Example Applications of Exposure-based Assessment of Joint Toxic Action for Carcinogenic Effects of Chemical Mixtures

The following examples are hypothetical examples chosen to illustrate how the procedures outlined in Figure 3 can be applied to a variety of exposure situations. Each example is for a single pathway and duration (assume intermediate or chronic) of exposure. The first three examples also were presented under Section 4.2.2 because they apply to the assessment of both noncarcinogenic and carcinogenic effects.

4.2.4.1. Residential Soil Contamination with CDDs and CDFs

Under Step 1, the ATSDR website is searched for relevant information, and the draft policy guideline for dioxin and dioxin-like compounds in soil (De Rosa et al. 1997a) is identified and downloaded. Further investigation locates the final policy guideline published as an appendix to the CDDs profile (ATSDR 1998b). This policy guideline provides the necessary guidance for health effects assessment of these mixture components; the guideline applies to carcinogenic effects (and to noncarcinogenic effects). Additional background can be obtained from the supporting documentation (De Rosa et al. 1997b, 1997c) and the toxicological profiles on CDDs and CDFs (ATSDR 1994, 1998b).

4.2.4.2. Groundwater Contamination with Chemicals A, B, and C

An interaction profile is available on this particular common mixture and can be identified by searching the ATSDR website. The interaction profile provides specific guidance on a health assessment approach for carcinogenic (and noncarcinogenic) effects of this mixture. Use the recommended approach to conduct exposure-based assessment of joint toxic action to screen for potential health hazard of the mixture, and use the ATSDR guidance for public health assessment (1992) for the other aspects of public health assessment.

4.2.4.3. Residential Soil Contamination with Toxaphene

Although no policy guideline or interaction profile (Step 1) is available for this mixture, a toxicological profile is available (Step 2), and provides risk-specific doses, a slope factor, and a unit risk for carcinogenic effects of toxaphene (from EPA), as well as MRLs for noncarcinogenic effects of toxaphene assessed as a whole mixture. These values and other information in the profile are used in accordance with ATSDR guidance for public health assessment (ATSDR 1992).

4.2.4.4. Groundwater Contamination with Chemicals D, E, F, and G

No policy guideline, interaction profile, or toxicological profile is identified for this mixture (Steps 1 and 2, Figure 3), but toxicological profiles are available for the individual chemicals, all of which have carcinogenic effects. A components approach is therefore initiated. The following four "cases" are hypothetical and are presented to illustrate the use of the approach in Figure 3 for mixtures with a relatively small number of components. It is assumed for the purposes of illustration that the point of departure for risk levels considered to have a significant impact on lifetime cancer risk is $1x10^{-4}$.

Case 1: The increased lifetime cancer risks for the individual components are estimated by multiplying the exposure dose by the slope factor.

chemical D:	exposure dose x slope factor	= 0.0001 mg/kg/day x $(1.0x10^{-3})$ $(mg/kg/day)^{-1}$	= $1x10^{-7}$
chemical E:		= 0.000043 mg/kg/day x $(4.7x10^{-4})$ $(mg/kg/day)^{-1}$	= $2x10^{-8}$
chemical F:		= 0.016 mg/kg/day x $(3.1x10^{-2})$ $(mg/kg/day)^{-1}$	= $5x10^{-4}$
chemical G:		= 0.003 mg/kg/day x 1.1 $(mg/kg/day)^{-1}$	= $3x10^{-3}$

Thus, the increased lifetime cancer risks for the components are $1x10^{-7}$, $2x10^{-8}$, $5x10^{-4}$, and $3x10^{-3}$. The first two component risks are below $1x10^{-6}$, and therefore are not expected to have a significant impact due to additivity or interactions. These two components are dropped from further consideration (Step 3). An applicable PBPK/PD model is not available for the components of concern (F and G) (Step 4). The sum of the risks for chemicals F and G results in a total risk of $3.5x10^{-3}$, which is rounded to $4x10^{-3}$, indicating that the mixture poses a *potential* health hazard due to additivity (Step 5). The qualitative WOE is applied to assess the potential impact of interactions (Step 6b). The BINWOE determinations are negative with a sum of -1.21. Thus, the potential health hazard is likely to be less than indicated by the sum of the cancer risks. Nevertheless, considering that the sum of the risks is significantly higher than the $1x10^{-4}$ point of departure, further evaluation using the methods in ATSDR (1992) is needed.

Case 2a: The estimated increased lifetime cancer risks (calculated in a manner similar to that in Case 1) are $1x10^{-7}$, $9x10^{-6}$, $3x10^{-5}$, and $5x10^{-5}$ for chemicals D, E, F, and G. The risk for chemical D is below $1x10^{-6}$, so this chemical is dropped from further consideration (Step 3). The estimated risks for chemicals E, F, and G are above $1x10^{-6}$, so these components are retained for further evaluation. No PBPK/PD or PBPK model is available for this three-component mixture, but a PBPK model is available for a binary mixture of chemicals E and F, and is applicable to oral exposure (Step 4). The model will be

considered subsequently during the evaluation of interactions for this pair. The sum of the risks for components E, F, and G is $9x10^{-5}$ (rounded from $8.9x10^{-5}$) (Step 5). The qualitative WOE method is used to assess the potential impact of interactions (Step 6a). BINWOEs for carcinogenic effects for the three possible pairs are available and are pertinent to carcinogenic effects. The BINWOEs for chemicals E and F have taken into account the PBPK model. The majority of the BINWOEs for the pairs in this mixture are greater than additive (positive) and a few are additive (0). Consistent with Step 6a, and considering that the total risk for the mixture is close to $1x10^{-4}$, it is concluded that the mixture constitutes a *potential* health hazard. Further evaluation using the methods in ATSDR (1992) is necessary.

Case 2b: Identical to Case 2a, except that, in Step 6a, the six BINWOEs for the three possible pairs are mainly less than additive (negative) and a few are additive (0). Because the BINWOEs indicate that the hazard is likely to be less than the sum of the risks, which in turn is less than $1x10^{-4}$, it is concluded that the mixture is unlikely to be a health hazard at the waste-site specific exposure levels (Step 6a).

Case 2c: Identical to Case 2a, except that the six BINWOEs for the three possible pairs are fairly evenly divided among greater than additive (positive), additive (0) and less than additive (negative). The sum of the BINWOE numerical scores is negative due to the greater strength of the evidence for the less-than-additive interactions. Consistent with Step 6a for a mixture with total risk less than $1x10^{-4}$, this result indicates that the mixture is unlikely to be a health hazard the health hazard at the estimated levels of exposure.

4.2.4.5. Air Contamination with Chemicals H, I, and J

No interaction profile or guidance policy is available for this mixture, but toxicological profiles and cancer inhalation unit risks are available for the individual components. Increased lifetime cancer risks for these chemicals are estimated by multiplying the exposure concentrations (converted to $\mu g/m^3$ if necessary) by the inhalation unit risks as follows:

chemical H:	exposure concentration x unit risk	= 0.2 $\mu g/m^3$ x $(3.2x10^{-4})$ $(\mu g/m^3)^{-1}$	= $6x10^{-6}$
chemical I:		= 0.006 $\mu g/m^3$ x $(1.8x10^{-3})$ $(\mu g/m^3)^{-1}$	= $1x10^{-5}$
chemical J:		= 0.05 $\mu g/m^3$ x $(8.4x10^{-5})$ $(\mu g/m^3)^{-1}$	= $4x10^{-6}$

Risks for all three chemicals are greater than $1x10^{-6}$ (Step 3). PBPK or PBPK/PD models are not available for the whole mixture or for the pairs of components (Step 4). The sum of the risks is $2x10^{-5}$ (Step 5). Following the procedures in Step 6, a qualitative WOE evaluation is undertaken. BINWOEs

for all three pairs, obtained from ATSDR, are additive (0) or less than additive (negative). BINWOEs for the effects of another component of concern, identified during the assessment of noncarcinogenic effects, on the carcinogenicity of these three chemicals are less than additive or indeterminate. Therefore, it is considered unlikely that exposure to these components in combination at the site-specific exposure levels will constitute a health hazard, although there is some uncertainty due to the indeterminate BINWOEs.

4.2.4.6. Groundwater Contamination with 12 Chemicals

No interaction profile or guidance policy is available for this mixture, but toxicological profiles and cancer slope factors or other cancer-based comparison values are available for three of the components (the others are not considered carcinogenic, but six of these other chemicals are considered components of concern for noncarcinogenic effects). One component, with an estimated cancer risk of $1x10^{-8}$, is dropped from further consideration (Step 3). Risks for the other two components are $1x10^{-6}$ and $3x10^{-6}$; these components are retained as components of concern (Step 3). PBPK or PBPK/PD models are not available for this mixture or for any of the pairs of chemicals within the mixture (Step 4). BINWOEs for the pair of carcinogenic components of concern are greater than additive, and for the effects of the other six components on the two carcinogenic components are a mixed pattern. The sum of the BINWOE numerical scores (combined score) is +0.14. This value is so close to zero that it does not significantly raise concern for greater-than-additive interactions, and the mixture is considered unlikely to be a health hazard at these levels of exposure.

4.3. MULTIPATHWAY EXPOSURE

If the same receptor subpopulation or individual can reasonably be expected to be exposed to site-related chemicals through more than one pathway, the hazard quotients, hazard indexes, and risks for a given duration can be summed across pathways to give the total hazard quotient, hazard index, and risk. Alternatively, the procedure outlined by Mumtaz et al. (1995) for estimating total integrated exposure and total tolerable levels could be explored.

4.4. NON-SITE-RELATED EXPOSURES AND MULTIPLE STRESSORS

The strategy for exposure-based assessment of joint toxic action of chemical mixtures described in Section 4.2 focuses on chemical mixtures associated with hazardous waste sites. As mentioned in the overview to this manual, additional non-site-related exposures also may be occurring to a variety of chemicals such as those in alcohol, tobacco, medicines, foods, vehicle exhaust fumes, drinking water, and

in the workplace. Information regarding these additional exposures can be taken into account during interpretation of the community-specific health outcome data and biomedical evaluation (ATSDR 1992). This information also may be helpful identifying populations that may be unusually sensitive to site-related chemicals, due to other chemical exposures. Similarly, populations exposed to physical, psychological, or biological stressors may be more susceptible to chemical insult to the body, as is suspected for some veterans of the Persian Gulf War (Yang 2000).

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APPENDIX A

BACKGROUND INFORMATION ON THE ASSESSMENT OF ADDITIVITY AND INTERACTIONS

A.1. INTRODUCTION

The approaches to assessing the joint action of components of a mixture are based in large measure on the conceptual groundwork laid by Bliss (1939) and Finney (1971), and are mathematical rather than biological in nature. The approaches commonly known as dose addition and response addition, discussed in the following sections, are non-interactive forms of joint action that assume the chemicals in the mixture do not affect the toxicity of one another, i.e., that they act independently. These assumptions are the bases for methods of risk and health assessment discussed in the Guidance Manual. In addition, the assessment of interactions depends on being able to define what constitutes non-interaction.

The available studies of toxicological interactions often pose a problem for the health assessor because the results may be ambiguous, often due to poor study design, or the results of several studies on the same mixture may appear to be conflicting, or the relevance of the study or studies to the exposure scenario of interest is uncertain. Approaches for dealing with these uncertainties are introduced in this appendix and further discussed in Appendices B and C.

A.2. MODELS FOR JOINT ACTION

A.2.1. DOSE ADDITION

As introduced in the Guidance Manual, dose addition, also known as concentration addition, simple similar action, and similar joint action, assumes that the components of a mixture behave as concentrations or dilutions of one another, differing only in their potencies (Bliss 1939; Finney 1971). The dose-response curves are parallel (i.e., the regression lines of probits on log doses are parallel), and tolerance (or susceptibility) to the components is completely positively correlated (the organisms most susceptible to chemical A also will be most susceptible to chemical B). The response to the mixture can be predicted by summing the doses of the components after adjusting for the differences in potencies. Dose addition is considered most appropriate for mixtures with components that affect the same endpoint by the same mechanism of action EPA (1986, 1990, 1999). It has been suggested that the requirement for parallel dose-response curves and complete correlation of tolerances may be too stringent (e.g.,

Plackett and Hewlett 1952; Svendsgaard and Hertzberg 1994), and that in the low-dose region in which the response is linear, dose additivity may hold for independently-acting chemicals as well (Svendsgaard and Hertzberg 1994). Dose addition is the underlying assumption of the hazard index method and the toxic equivalency factor (TEF) approach (Sections 2.3.1 and 2.3.3).

The regression lines for two chemicals (1 and 2) that act in a dose additive manner can be represented as:

$$Y_1 = \beta \log x + \alpha_1 \quad \textbf{(1)}$$

$$Y_2 = \beta \log x + \alpha_2 \quad \textbf{(2)}$$

where x is dose or concentration, Y_i is the probit response for the i^{th} chemical, β is the slope (by definition the same for both chemicals), and α_i is the intercept on the exposure axis (the value of Y when x is zero) for the i^{th} chemical. The potency ρ of chemical 2 relative to chemical 1 is:

$$\log \rho = \frac{(\alpha_2 - \alpha_1)}{\beta} \quad \textbf{(3)}$$

Using equation 3 to convert the dose of the second chemical into an equivalent amount of the first, equation 2 can be rewritten as:

$$Y_2 = \beta \log(\rho \cdot x) + \alpha_1 \quad \textbf{(4)}$$

Thus, for a mixture of chemicals 1 and 2 in which the exposures are x_1 and x_2, the response is dose additive if it equals that produced by a dose $(x_1 + \rho \cdot x_2)$ of the first chemical alone, as expressed by the following equation:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \cdot x_2) \quad \textbf{(5)}$$

Alternatively, if the mixture is regarded as a total dose x, in which the proportions of the two chemicals are π_1 and π_2, equation 5 can be written as:

$$Y = \alpha_1 + \beta \log(\pi_1 + \rho \pi_2) + \beta \log x \quad \textbf{(6)}$$

Equations 5 and 6 can be generalized for a greater number of components.

Relationships that may be useful in analyzing interactions data (Finney 1971) can be derived from equation 6. If for a mixture of defined proportions of chemical 1 and 2, some uniform measure of toxicity (risk-specific dose or equally effective dose, e.g., ED_{50}) is known for the two chemicals and designated by ζ_1 and ζ_2, respectively, then:

$$\zeta_2 = \frac{\zeta_1}{\rho} \quad \textbf{(7)}$$

The toxicity ζ_m of any mixture of chemicals 1 and 2 can be predicted as follows under the assumption of dose addition:

$$\zeta_m = \frac{\zeta_1}{(\pi_1 + \rho\pi_2)} \quad \textbf{(8)}$$

Equation 8 can also be written in the following form:

$$\frac{1}{\zeta_m} = \left(\frac{1}{\zeta_1}\right)\pi_1 + \left(\frac{\rho}{\zeta_1}\right)\pi_2 \quad \textbf{(9)}$$

Based on equation 7, $1/\zeta_2$ can be substituted for ρ/ζ_1 in equation 9 to give:

$$\frac{1}{\zeta_m} = \frac{\pi_1}{\zeta_1} + \frac{\pi_2}{\zeta_2} \quad \textbf{(10)}$$

This form of the equation can be used to predict the ED_{50} (or other uniform measure of toxicity) of a mixture from the proportions and ED_{50}s of the components.

A.2.2. APPLICATIONS OF DOSE ADDITION TO HEALTH AND RISK ASSESSMENT

The toxic equivalency (TEQ) approach and hazard index approach are based on the assumption of dose addition. The response to the mixture is considered dose additive if it equals that produced by a dose of the first chemical alone. The mixture dose (X), expressed as an equivalent dose of the first chemical alone, is:

$$X = \rho_1 x_1 + \rho_2 x_2 + \rho_3 x_3 + \cdots + \rho_n x_n \quad \textbf{(11)}$$

where ρ_i is the potency of the i^{th} component relative to the first chemical and x_i is the concentration or dose of the i^{th} component. Note that $\rho_1 = 1$, the potency of chemical 1 relative to itself.

In the TEQ approach, the first or index chemical is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which is assigned a toxic equivalency factor (TEF) of unity, representing its potency relative to itself. TEFs for the other active congeners are based on their potency relative to 2,3,7,8-TCDD. The concentrations or doses of all active congeners are multiplied by their TEF values and summed to give the *TEQs* for the mixture, which is the concentration of the mixture expressed as an equivalent concentration of the index chemical, 2,3,7,8-TCDD:

$$TEQs = TEF_1 C_1 + TEF_2 C_2 + TEF_3 C_3 + \cdots + TEF_n C_n = \sum_{i=1}^{n} TEF_i C_i \qquad \textbf{(12)}$$

where TEF_i is the potency of the i^{th} component relative to 2,3,7,8-TCDD and C_i is the concentration of the i^{th} component (ATSDR 1998; EPA 1994; Van den Berg et al. 1998). Equation 12 is equivalent to equation 5 of the Guidance Manual. The relative potency method for polycyclic aromatic hydrocarbons (PAHs) (ATSDR 1995; EPA 1993) is a similar application of dose addition. Additional information and references are provided in Section 2.3.4 of the Guidance Manual.

The hazard index approach uses 1/DL (where DL is a defined level of exposure such as an MRL or RfD) as an indicator of potency (because the larger the DL, the less the potency) for the components of a mixture. If E is the total mixture dose or exposure expressed as the equivalent dose of chemical 1, where chemical 1 can be any component of the mixture, then, under dose addition:

$$E = \frac{DL_1}{DL_1} E_1 + \frac{DL_1}{DL_2} E_2 + \frac{DL_1}{DL_3} E_3 + \cdots + \frac{DL_1}{DL_n} E_n \qquad \textbf{(13)}$$

where DL_i is the defined level for the i^{th} component, and E_i is the exposure to the i^{th} component, in the same units. Factoring out DL_1 from the numerators, equation 13 becomes:

$$E = DL_1 \left(\frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n} \right) \qquad \textbf{(14)}$$

Dividing both sides of equation 14 by DL_1 gives the expression for the hazard index (HI):

$$\frac{E}{DL_1} = HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n} \quad \textbf{(15)}$$

The hazard index approach is discussed further in Section 2.3.1 of the Guidance Manual.

Limitations of the hazard index approach include the requirement imposed by the dose addition model that the mode of action of the chemicals be similar, and the weakness of the assumption that the defined levels (MRLs or RfDs) represent isoeffective doses. Potential improvements to the approach include the use of toxicity thresholds or effective dose levels (e.g., ED_{10}s), rather than MRLs or other defined levels, but there are analytical problems in determining these values as well, and they are not available for most chemicals. Svendsgaard and Hertzberg (1994) have discussed the statistical issues associated with the hazard index approach.

A.2.3. RESPONSE ADDITION

Response addition, as introduced in the Guidance Manual (Section 2.3), *Response Addition*, also known as simple independent action and independent joint action (Bliss 1939), assumes that the chemicals act independently and by different modes of action. Because the modes of action are different, tolerance (or susceptibility) to the components is not necessarily positively correlated under response addition. The response to the mixture can be predicted from the responses to the components and the correlation of tolerances. Response addition is the underlying assumption of an approach to cancer risk assessment for mixtures and ACGIH's approach to assessing the hazard of occupational exposure to agents that act independently (Sections 2.3.5 and 3.1).

The form of response addition will be different depending on the correlation of susceptibility to the components of the mixture. If the organisms most sensitive to chemical 1 are also most sensitive to chemical 2, susceptibilities to chemicals 1 and 2 are completely and positively correlated. The correlation coefficient r is equal to one. The expected response P to the mixture of chemicals 1 and 2 at doses that individually produce responses P_1 and P_2 is equivalent to that for the chemical with the highest response. Thus:

$$\begin{aligned} P &= P_1 \quad \text{if } r = 1 \quad \text{and} \quad P_1 > P_2 \\ P &= P_2 \quad \text{if } r = 1 \quad \text{and} \quad P_2 > P_1 \end{aligned} \quad \textbf{(16)}$$

In other words, if the dose of chemical 1 would be expected to cause a response in 8% of the animals and chemical 2 would be expected to cause a response in 17% of the animals, the expected response to the mixture of these two chemicals at these doses is 17% when susceptibilities are completely positively correlated.

If the organisms most sensitive to chemical 1 are least sensitive to chemical 2 and vice versa, susceptibilities to chemicals 1 and 2 are completely and negatively correlated. Under this circumstance, the predicted response to the mixture would be simply additive (8 + 17 = 25%) as long as the total of the responses to chemicals 1 and 2 was less than unity.

$$P = P_1 + P_2 \quad \textit{if } r = -1 \quad \textit{and} \quad (P_1 + P_2) \leq 1 \tag{17}$$

Intermediate to these two extremes is the circumstance when the susceptibility to the two chemicals are statistically independent. In this case, some of the organisms that would not respond to chemical 1 would respond to chemical 2, so that the total response rate for the mixture is:

$$\begin{aligned} P &= P_1 + P_2(1 - P_1) \\ &= P_1 + P_2 - P_1P_2 \end{aligned} \tag{18}$$

Using the same response rates as in the previous examples, the response to the mixture would be estimated as $100(0.08 + 0.17 - (0.08 \cdot 0.17)) = 23.6\%$.

The above equations can be generalized for a greater number of components.

A.2.4. APPLICATIONS OF RESPONSE ADDITION TO HEALTH OR RISK ASSESSMENT

The relationships of the equations for the various forms of response addition to their applications in risk assessment are more intuitively obvious than is the relationship of the equations for dose addition to such applications as the hazard index. Accordingly, the applications will not be discussed in detail here, but rather mentioned with a reference to the section of the Guidance Manual in which they are presented.

An approach similar to response addition assuming completely positive correlation of tolerances (equation 16 of this appendix) has been applied by ACGIH to the assessment of mixtures whose components are expected to cause effects that are independent from each other, such as purely local effects on different organ systems. The threshold limit for the mixture is considered to be exceeded only if the hazard quotient for at least one of the components exceeds unity (Section 3.1).

The calculation of total cancer risk (Section 2.3.5) is based on response addition with completely negative correlation of tolerances. The responses (risks) for the individual components of the mixture are summed to estimate the response to the mixture as in equation 17 of this appendix.

A.3. INTERACTIONS

A.3.1. INTRODUCTION TO INTERACTION MODELS

The assessment of interactions involves assumptions regarding what constitutes an additive or non-interactive response. Thus, the assumed form of additivity often drives experimental design and the assessment of joint action. Knowledge of the mode of action of the individual components of the mixture is often used in selecting a plausible additivity model.

If interactions appear to exist, as determined from deviations from the assumed form of additivity, mathematic models for quantifying the interactions may be used. Finney (1942, 1971) proposed the following interaction model, which is a modification of equation 5 for dose addition:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \cdot x_2) + \kappa(\rho \cdot x_1 \cdot x_2)^{0.5} \quad \textbf{(19)}$$

where κ is the interaction coefficient. Positive values of κ indicate synergism, negative values indicate antagonism, and a value of zero indicates dose addition.

A.3.2. EXPERIMENTAL STUDIES

Experimental studies of toxicological interactions, particularly those designed primarily to investigate the mechanism of action of the chemical of interest, may not reflect the models discussed above. From the material already presented in this appendix, it follows that, in general, an understanding of the joint action of the components of a mixture depends upon an understanding of the dose-response relationships for the individual components. There are exceptions to this generalization. An example is the case where one component is known to be inactive with regard to the effect of concern. In this case, only the dose-response curves for the active component with and without the addition of the inactive component may be necessary.

Other interaction studies do use dose addition or response addition models in the evaluation of additivity versus interactions. For example, Smyth et al. (1969) used equation 10 to predict the toxicity (LD_{50}) of

the 350 possible binary mixtures of 27 industrial chemicals administered in equivolume combinations. (One pair of chemicals proved impossible because it reacted vigorously upon mixing before administration.) The ratio between the predicted (*P*) and observed (*O*) values, calculated for each pair, ranged from 0.23 to 5.09, indicating that the magnitude of deviation from dose additivity was approximately a factor of 5 or less. This is not a remarkable deviation from additivity and thus suggests that dose additivity is a reasonable default model for joint action. The upper end of the range of the deviation from additivity of 5 also has been used as the basis for a default "magnitude of interaction" factor in the modified WOE method (EPA 1999) described in Appendix B. Smyth et al. (1970) retested 53 chemical pairs from this set in equitoxic combinations. Because the distribution of ratios for the first (equivolume) study was skewed, the investigators normalized the ratios in that study and in the equitoxic study using the following adjustment:

where $P/O>1$; adjusted ratio = $(P/O) - 1$
where $P/O<1$; adjusted ratio = $1 - (O/P)$

With the adjusted ratios, a positive value indicates greater-than-additive joint action, a negative value indicates less-than-additive joint action, and a value of zero indicates additivity.

The equivolume and equitoxic experiments used different proportions of the chemicals for each pair. The difference in proportions should not affect the ability of equation 10 to predict the LD_{50} for the mixture. A comparison of the adjusted ratios in the equivolume and equitoxic experiments on the same pairs of chemicals showed that the correlation between the two sets of ratios was good. These results further support dose addition as a reasonable default model for joint action.

Further guidance regarding the evaluation of studies of joint toxic action is provided in ATSDR (2001).

A.3.3. ASSESSING THE RELEVANCE OF INTERACTIONS STUDIES TO HUMAN HEALTH

Much of the information available on toxicological interactions is for binary mixtures of chemicals. Most of the studies summarized in MIXTOX (EPA 1990), a database that focuses primarily on interactions relevant to noncancer toxicity, are for short durations of dosing. A large proportion of the studies in this database used a sequential rather than simultaneous exposure protocol, and the great majority focused on lethality or liver toxicity as an endpoint. When two or more studies were available on a particular binary mixture, the results were sometimes conflicting and the experimental variables different. Interpretation of this information is problematic when the objective is to predict the potential

impact on public health from exposure to a mixture consisting of more than two chemicals, where exposure to these chemicals is occurring simultaneously, for extended durations, and at relatively low doses. Similar conclusions as to the relevance of the available interactions data to human health have been reached by Krishnan and Brodeur (1991) in their monumental review of interactions studies on both noncarcinogenic and carcinogenic endpoints.

Methods for predicting joint toxic action from this type of data include the Weight-of-Evidence (WOE) methods (EPA 1999; Mumtaz and Durkin 1992; Mumtaz et al. 1994) discussed in Appendix B and the Integral Search System (ISS) (DiCarlo and Woo 1994; Woo et al. 1994) discussed in Appendix C. The WOE methods require a careful evaluation of the available interactions data, supplemented by the evaluation of mechanistic, pharmacokinetic, and toxicological data, plus a consideration of structure activity relationships—for all binary combinations of chemicals in a mixture of concern. This degree of analysis may pose a problem in terms of the numbers of chemical pairs that would be of interest for mixtures associated with hazardous waste sites.

Potential solutions to this problem are likely to involve computer programs that perform the analyses automatically. One solution, offered by ISS, is to count, for each pair of chemicals, one "hit" if one (or more) studies have reported an interaction in an interaction category scored by that program, sum the hits in each category for all possible pairs, and compute a composite score for the mixture, weighted for the estimated importance of a given interaction category (such as synergism). A chemical pair with 6 studies showing synergism, 0 for promotion, 0 for antagonism, and 1 for inhibition would have a score of 1 for synergism, 0 for promotion, 0 for antagonism, and 1 for inhibition. The ISS also takes into account the potential interactions for a chemical without data by assessing the interactions of the structural or functional class to which the chemical belongs. It then uses the numbers of hits along with a weighting factor to calculate a "weighting ratio" that reflects the potential impact of interactions on the hazard of the mixture. The limitations of ISS are discussed in Appendix C.

Another potential solution is to develop ways to count each result in each interaction category (synergism, additivity, antagonism) for each pair of chemicals, and assess the variance of results and the statistical significance of the observed pattern. This method, developed by Durkin et al. (1995), based on the data in MIXTOX, can be used to assess the patterns of interactions between single chemicals, a chemical and a class, or between classes of chemicals. In addition, it can be used to define a class of chemicals based on empirical similarities, i.e., a class consisting of chemicals that appear to interact in a similar manner with one or more other chemicals. Significant interaction patterns for classes of chemicals could be used as "rules" for chemicals in those classes that lack interactions data, in support of

WOE assessments. A limitation of this study was the paucity and variability of the interactions studies on any given pair of chemicals (data used for these analyses were current through 1991). Given the increased interest in the toxicological interactions of environmental contaminants, it is possible that considerably more data may be available now to support the patterns approach, making further development worthwhile.

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APPENDIX B

WEIGHT-OF-EVIDENCE METHODS

B.1 INTRODUCTION

The weight-of-evidence (WOE) methods for the assessment of chemical interactions described in this appendix were designed to facilitate the use of interactions data in the components-based assessment of noncancer health effects from exposure to chemical mixtures. As noted above, the hazard index method does not incorporate information on interactions among components of the mixture. A WOE method proposed by Mumtaz and Durkin (1992) was the first systematic attempt to address this need. The method implemented and expanded on the suggestion of the NRC (1989) that an uncertainty factor be used to account for interactions among components of a mixture. The value of the uncertainty factor can reflect the concern for interactions, and is modified using data regarding the WOE for interactions (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). As suggested by the NRC, the uncertainty factor is applied to the additivity-based hazard index to estimate an interactions-adjusted hazard index. Subsequent experience with the algorithm that is used to generate the interactions-adjusted hazard index has revealed, however, that it does not handle changes in the proportions of mixture components in a reasonable manner. The method remains useful in the qualitative prediction of whether hazard may be greater or less than indicated by the hazard index (Sections B.1.2 and B.2.2).

A modification to the WOE method was developed (ERG and Durkin 1995; EPA 1999) in order to explicitly incorporate information on the magnitudes of the pairwise interactions into the risk assessment. This modified method addresses some of the limitations of the original method, but introduces a new set of limitations: greater judgment may be required in the scoring of the weight-of-evidence and information on the magnitude of interactions is rarely available.

An abbreviated description of the original method was presented in the guidance manual; some of the information will be repeated here for the sake of completeness and to facilitate comparison of the two methods. The following sections provide additional details of these methods.

B.2 ORIGINAL WOE METHOD

B.2.1. BINARY WEIGHT OF EVIDENCE SCORES

The first step in applying the WOE method is to assess data relevant to joint action for each possible pair of chemicals in the mixture in order to make a qualitative binary weight-of-evidence (BINWOE) determination for interactions. The BINWOE determination is a classification that reflects the quality of the available information and categorizes the most plausible nature of the potential influence of one chemical on the toxicity of another chemical for a given exposure scenario (duration, route, and sequence). This determination includes evaluating information regarding the toxicity, pharmacokinetics, and mechanism of action of the individual chemicals; interactions data on each chemical pair; and interactions and mechanistic data on related chemicals. Although the earlier publications of the WOE method did not discuss the need for BINWOE determinations to take into account target organ (Durkin 1995; Mumtaz and Durkin 1992), experience in application of the WOE method has indicated that the WOE evaluations should be target-organ specific (Mumtaz et al. 1998). Two BINWOE determinations are made for each pair: one for the effect of chemical A on the toxicity of chemical B, and the other for the effect of chemical B on the toxicity of chemical A (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). The criteria and scoring system for the BINWOE determinations are presented in Table B-1.

The classification of direction of interaction in Table B-1 has the following categories: additive, greater-than-additive, less-than-additive, and indeterminate. The additive category refers to results that are additive by a defined model of additivity (e.g., dose or response addition), and results which demonstrate no effect of one chemical on the toxicity of the other. The greater-than-additive category refers to synergism or potentiation. The less-than-additive category refers to antagonism, inhibition, or masking. Indeterminate refers to instances of ambiguous, conflicting, or no data.

The classification of the quality of the data in Table B-1 includes two main categories: mechanistic understanding and toxicological significance. The rating for mechanistic understanding reflects the quality of the available mechanistic data supporting a toxicological interaction and the extent to which this information indicates the direction of the interaction. Mechanistic information is information regarding the manner in which a chemical causes a given toxic effect or interaction, and may include chemical, biological, and physical processes at the molecular level and at higher levels of biological or physiological organization. The rating for toxicological significance reflects the quality of the available toxicological interactions data and the extent to which it indicates that the chemicals will interact in a manner that significantly impacts the health of the exposed population. Both the mechanistic and

toxicological categories allow for, and encourage, the use of structure-activity data in reaching conclusions. The "modifiers" in Table B-1 are used when the mechanistic and toxicological ratings do not account for the additional concerns for differences in duration, sequence, bioassay (*in vitro* versus *in vivo*), or route of exposure between the site-specific exposures and the mechanistic and toxicological data used for the BINWOE determinations (Mumtaz and Durkin 1992).

The qualitative direction and alphanumeric data quality terms are shown in the left column of Table B-1. The corresponding direction factor and numerical data quality weighting factors are shown in the right column. The qualitative scores can be converted to a single numerical score by multiplying the direction factors (labeled "Direction" in the table) and the data quality weighting factors (labeled "Weight" in the table). Thus, an alphanumeric (qualitative) BINWOE classification of >II.B.2.a.i corresponds to greater-than-additive interaction, mechanistic data on related chemicals, inferred toxicological significance, different duration or sequence, *in vivo* data, and anticipated route of exposure. The corresponding numerical BINWOE score is +1(0.71)(0.71)(0.79)(1)(1) = +0.40.

The data quality weighting factors were selected using the following reasoning: the optimum score for data quality is unity, and corresponds to the first level of scoring (categories I and A for the primary classifications of mechanistic or toxicological significance and 1, a, and I for the modifiers). For the primary classifications, the value of 0.71 was selected for the second level of scoring (categories II and B) so that if both factors were selected the score would be about one-half of the optimum score ($0.71 \cdot 0.71 \approx 0.50$). Similarly, for the third level of scoring (categories III and C), the value of 0.32 was selected so that if both factors were selected the score would be about one-tenth of the optimum score ($0.32 \cdot 0.32 \approx 0.1$). For the modifiers, a value of 0.79 was selected for the second level of scoring (2, b, and ii) so that all three factors combined would lower the score by a factor of about 0.5 ($0.79 \cdot 0.79 \cdot 0.79 \approx 0.5$). The numerical weighting values reflect judgment as to the relative importance of the data quality classifications in determining the weight of evidence (Durkin 1995).

The BINWOE determinations do not explicitly consider the relevance of dose to the anticipated exposure scenario. It is not uncommon to find that, for a well-studied binary mixture, the available information suggests that no interactions occur at low doses, but that an interaction, either greater-than-additive or less-than-additive, occurs at higher doses. The BINWOE for this situation would reflect the interaction observed at higher doses. Dose *is* taken into account in the calculation of interaction factors (Section B.2.2). Additional guidance for the determination of BINWOEs is provided in the ATSDR (2001) *Guidance for the Preparation of an Interaction Profile*.

Table B-1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

	Classification	Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	-1
?	Indeterminate	0
Quality of the Data		**Weighting**
	Mechanistic Understanding	
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur have not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
	Toxicological Significance	
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
	Modifiers	
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79
Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05		
BINWOE = Direction Factor x Weighting Factor: Ranges from –1 through 0 to +1		

*Adapted from Mumtaz and Durkin (1992) and Mumtaz et al. (1994a)

B.2.2. QUALITATIVE WOE METHOD

A qualitative WOE approach, focusing on application of the BINWOE scores to hazardous waste-site assessment, was suggested by Mumtaz and Durkin (1992). This approach is appropriate for a mixture where the scaled doses (hazard quotients) for all the components are similar, or toxicologically significant. The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2-N)/2$. Thus, this mixture of 4 components has 6 pairs of components and potentially 12 BINWOEs. Suppose nine of the BINWOEs are greater-than-additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the weight of evidence suggests that the mixture is likely to pose a greater hazard than that indicated by the hazard index.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater than additive, some less than additive, and some additive BINWOEs). In this case, the qualitative WOE approach is extended to include conversion of the qualitative BINWOE scores to numerical scores, and summing the scores to give a combined score. If the combined BINWOE score is positive and significantly different from zero, then the weight of evidence suggests that the mixture is likely to pose a greater hazard than indicated by the hazard index. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the weight of evidence suggests that the health hazard is unlikely to be greater than indicated by the hazard index. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index.

Although the above WOE method was developed for assessing interactions for noncarcinogenic effects, the qualitative WOE method is equally applicable to assessing interactions for carcinogenic effects.

B.2.3. INTERACTION FACTORS

The quantitative application of the WOE method is described in this section, and continues through Section B.2.5. As mentioned previously, this quantitative application does not handle changes in the proportions of mixture components in a reasonable manner, and is no longer in use. The description is retained in this document because the method represents an interesting and original attempt to modify the hazard index for interactions.

In this quantitative application, the BINWOEs are used as interaction terms in the calculation of interaction factors, $IF_{i,j}$ and $IF_{j,i}$ (where $IF_{i,j}$ is the effect of j on the toxicity of i and $IF_{j,i}$ is the effect of i on the toxicity of j) as follows:

$$IF_{i,j} = \frac{HQ_i}{HI_{add}} \cdot BINWOE_{i,j} \; (HQ_i \cdot HQ_j)^{0.5} \quad \textbf{(1)}$$

$$IF_{j,i} = \frac{HQ_j}{HI_{add}} \cdot BINWOE_{j,i} \; (HQ_i \cdot HQ_j)^{0.5} \quad \textbf{(2)}$$

The two equations are identical except that equation 1 calculates the interaction factor for the effect of j on the toxicity of i, and equation 2 calculates the interaction factor for the effect of i on the toxicity of j.

The first set of terms in these equations weights the interaction factor by the contribution of the chemical whose toxicity is affected to the total toxicity of the mixture, expressed as the ratio of the hazard quotient (HQ_i) of that chemical to the total additivity-based hazard index (HI_{add}) of the mixture (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). This approach is adapted from one developed by Durkin (1981) to account for asymmetrical interactions under the assumption of dose additivity. Asymmetrical interactions are those in which the magnitude and sometimes the direction of the interaction vary with the proportions of the components in the mixture. It should be noted that there is a slight difference between the algorithms in Mumtaz and Durkin (1992) and Mumtaz et al. (1994a). In the 1994 paper (Eq. 2a and 2b), the term $HQ_i/(HQ_i+HQ_j)$ is used. In the 1994 review (Mumtaz et al. 1994a), the term HQ_i/HI_{add} is used.

The BINWOE score is the interaction term, which quantifies concern with interaction for a chemical pair. Estimation of the BINWOE score was discussed in the previous section.

The last set of terms in these two equations is the geometric mean of the hazard quotients for the two chemicals. Finney (1942, 1971) proposed a similar term for modeling symmetrical interactions under the assumption of dose additivity. The use of the geometric mean lowers the value of the interaction factor as exposure to either of the two chemicals falls below the defined level (denominator of the hazard quotient, e.g., MRL) for that chemical, i.e., as either hazard quotient falls below unity. This property of the WOE approach is consistent with the general observation that as exposure levels and the probability of responses due to the individual components decrease, the toxicological significance of interactions in a mixture will decrease (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). In addition, the use of the geometric mean lowers the value of the interactions factor as the hazard quotients of the two components

deviate from each other. This is consistent with the assumption that the greatest departure from additivity (greatest interaction) will occur when both components of a binary mixture are present in equitoxic amounts. This assumption also is expressed in Finney's model of a deviation from dose additivity (Finney 1942, 1971), presented in Appendix A (Section A.3.1).

B.2.4. WOE

The next step in this method is to sum the interaction factors to express the overall direction and weight of evidence for the toxicological interactions of the site-specific mixture, WOE_S.

$$WOE_S = \sum_{i \neq j}\sum IF_{i,j} \quad \textbf{(3)}$$

The double summation sign indicates that each component of the mixture is evaluated for the effect that every other component could have on its toxicity. The overall process (substituting the full expression for the interaction factors into equation 3) can be represented by equation 4.

$$WOE_S = \sum_{i \neq j}\sum \frac{HQ_i}{HI_{add}} \cdot BINWOE_{i,j} \cdot (HQ_i \cdot HQ_j)^{0.5} \quad \textbf{(4)}$$

The WOE_S score has no absolute or clear interpretation. For example, a score of -0.16 could be a composite of interaction factors for antagonism (-0.223) and synergism (+0.060) or a composite of interaction factors all of which reflect very low confidence in antagonism (e.g., -0.01, -0.04, -0.05, -0.01, -0.02, -0.03). Therefore, Mumtaz and Durkin (1992) recommended that the WOE be normalized by dividing the WOE_S by the maximum possible score that the site-specific mixture would have generated if all the interactions information had indicated a consistent direction of interaction and had been assigned weighting scores indicating the highest possible degree of confidence (BINWOE determinations of I.A.1.a.i with corresponding BINWOE scores of 1.0). Because the BINWOE scores are 1, they essentially drop out of equations 1 and 2 for the interactions factors, and therefore out of equation 4. Accordingly, the maximum possible score, WOE_{MAX}, can be calculated by summing the simplified expressions for the interaction factors as follows:

$$WOE_{MAX} = \sum_{i \neq j}\sum \frac{HQ_i}{HI_{add}} \cdot (HQ_i \cdot HQ_j)^{0.5} \quad \textbf{(5)}$$

The normalized WOE for the site-specific mixture, WOE_N, is:

$$WOE_N = \frac{WOE_S}{WOE_{MAX}} \quad \textbf{(6)}$$

The WOE_N is an expression of the strength of the evidence suggesting that interactions may be toxicologically significant relative to the highest possible level of confidence that can be expressed for the site-specific mixture using this method. For example, consider the previously mentioned site-specific mixture with an estimated WOE_S of –0.16 (the sum of interaction factors indicating less-than-additive and greater-than-additive interactions). Suppose the WOE_{MAX} for this site is 0.75. The WOE_N is calculated as –0.16/0.75 = –0.21. Thus, the strength of the available data on the binary interactions, when used with the exposure data from the site, suggests that the net effect of interactions for the mixture is likely to be less-than-additive, as indicated by the minus sign in the WOE_S and WOE_N scores. Relative to (hypothetical) interactions data of the highest possible quality for the same mixture and exposures, overall confidence in the assessment of less-than-additive toxicity for this site-specific mixture is about 20%, as indicated by the magnitude of the WOE_N score (Mumtaz and Durkin 1992; Mumtaz et al. 1994a).

B.2.5. INTERACTIONS-BASED HAZARD INDEX

Consistent with the suggestion by the NRC (1989) that the hazard index be adjusted for interactions through the application of an uncertainty factor, and with EPA and ATSDR approaches to assessing the noncancer toxicity of individual chemicals, Mumtaz and Durkin (1992) suggest that the hazard index be adjusted for the uncertainty of interactions by the application of an uncertainty factor. The uncertainty factor is modified by the normalized WOE score, WOE_N. The adjustment is performed as follows:

$$HI_I = HI_{add} \times UF_I^{WOE_N} \quad (7)$$

where HI_I is the interactions-based hazard index, HI_{add} is the additivity-based hazard index, and UF_I is an uncertainty factor for interactions. Thus, the hazard index is multiplied by the uncertainty factor for interactions to the power of WOE_N.

The NRC (1989) discussed the use of an uncertainty factor in the range of 1 to 100 depending on the available interactions information and the concentrations of the components. Mumtaz and Durkin (1992) note that the value of the uncertainty factor UF_I could be set by taking into account the concern for the magnitude of an interaction, but that suitable data regarding magnitude generally are not available. For the purposes of illustration, an uncertainty factor of 10 has been used in the various examples and exercises performed with this WOE methodology. Because WOE_N can range from -1 (for the highest possible confidence in less-than-additive interactions) to +1 (for the highest possible confidence in greater-than-additive interactions), UF_I to the power of WOE_N can range from 0.1 to 10. The net effect can be to increase *or decrease* the hazard index by a factor of 10. The WOE approach therefore differs from the NRC (1989) approach, which uses an uncertainty factor only to increase the hazard index. It

also differs from ATSDR and EPA approaches to assessing the noncancer toxicity of individual chemicals through the derivation of MRLs, RfDs, and RfCs, in which uncertainty factors are applied to make the health criterion more conservative.

As an example of the application of the WOE method, the WOE_N of -0.21 discussed in the previous section and an additivity-based hazard index of 2 are substituted into equation 7 to estimate the interactions-based hazard index, as follows:

$$HI_I = 2 \cdot 10^{-0.22} = 1.2 \quad \textbf{(8)}$$

For a WOE_N of +0.22, and a hazard index of 2, the interactions-based hazard index would be 3.3. A larger value of WOE_N, +0.75, applied to a hazard index of 2 would result in an interactions-based hazard index of 11.

B.2.6. STRENGTHS AND LIMITATIONS OF THE ORIGINAL WOE METHOD

The highly prescriptive method for BINWOE classification is designed to encourage a consistent application of the methodology. The application was considered consistent by expert toxicologists who reviewed the results of exercises in which 5-6 teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals, using the same data (Mumtaz et al. 1994b).

The separation of mechanistic understanding from toxicological significance and equal weighting of the these two categories has been questioned on the grounds that mechanistic understanding is important in risk assessment only as it serves to support or modify toxicological significance. Based on analyses of interactions data, the sequence of exposure appears to have a more profound impact on the nature of the interaction than does route or possibly duration (Hertzberg and Durkin 1994). It has been suggested that the sequence of exposure be separated from duration and given a separate weighting factor to better reflect the impact of sequence on the nature of the interaction (Durkin 1995).

The algorithms do not provide a means for using information on the magnitudes of the interactions for specific pairs of components, should such information be available. Rather, the magnitudes of the interactions among the components of a mixture are represented by a single uncertainty factor, which is modified by the WOE determinations, and then applied to the hazard index. Given the scarcity of suitable data for determining the magnitude of interactions, this may not be a limitation. The normalization process was considered useful as an indicator of confidence in the assessment of direction

of interactions for the site-specific mixture and when there is a need to compare scores across hazardous waste sites. It also constrained the value of the interactions-modified uncertainty factor within reasonable limits (0.1 to 10).

The WOE method (Mumtaz and Durkin 1992; Mumtaz et al. 1994a) has undergone evaluation, and appeared to perform well qualitatively, and quantitatively under some circumstances. The application of the method for deriving BINWOE classifications was considered consistent by expert toxicologists who reviewed the results of exercises in which several teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals (Mumtaz et al. 1994b). In tests of the WOE method to predict the toxicity of some simple chemical mixtures to animals, BINWOEs for three pairs of chemicals qualitatively predicted whether the results of animal studies would be less-than-additive, additive, or greater-than-additive (Mumtaz et al. 1998). Used with an exponential dose-response model and dose addition to model relative kidney weights, the quantitative WOE method closely predicted the observed dose-response in female rats for intermediate-duration oral exposure to a mixture of four nephrotoxic chemicals with similar modes of action (Mumtaz et al. 1998). The observed dose-response was less than dose additive. The BINWOEs were focused on renal toxicity, and the uncertainty factor used in the algorithm was 10. The WOE method underestimated the relative liver weights in the same animals. The observed dose-response for relative liver weight was slightly greater than dose additive. Thus, the WOE method did not predict toxicity to a target organ that was different from the one for which the BINWOEs were derived. The WOE method slightly overpredicted the observed dose-response for relative kidney weight in male rats for a mixture of dissimilarly acting nephrotoxins (in female rats, the data variability was so great that the exponential model did not fit the *observed* responses) (Mumtaz et al. 1998). Although these results are suggestive, limitations of this test of the complete WOE method include the substantial variability in the responses of individual animals, small numbers of animals per group, testing of only two dose levels of the mixtures, and lack of rationale for using relative organ weight as an index of toxicity (several other indicators of renal and hepatic toxicity were monitored in the studies that provided the experimental data [Jonker et al. 1993, 1996]).

Subsequent experience with the WOE method revealed, however, that the algorithm does not handle changes in proportions of mixture components in a reasonable manner. Therefore, ATSDR has discontinued the use of the algorithm and will use a qualitative WOE approach (Section B.2.2), as suggested by Mumtaz and Durkin (1992), until an appropriate algorithm can be developed or selected, and fully evaluated. The WOE algorithm and other approaches of this type must be tested to ensure that they behave in a reasonable and consistent manner with regard to the underlying assumptions and that their predictions are reasonable representations of experimental or known exposure outcomes.

B.3. MODIFIED WOE METHOD

B.3.1. MODIFIED BINARY WEIGHT OF EVIDENCE SCORES

The modified WOE method, proposed by ERG and Durkin (1995), further developed by EPA, and adopted as part of EPA (1999) mixtures guidance, employs an alternative weight-of-evidence classification scheme that focuses on a more integrated interpretation of the data. The suggested numerical weights for the various classifications range from 0 to 1.0 as in the original methodology. As in the original method, two BINWOE determinations are made for each pair: one for the effect of chemical A on the toxicity of chemical B, and the other for the effect of chemical B on the toxicity of chemical A. Unlike the original methodology, less weight is given to less-than-additive interactions under circumstances where there is some uncertainty regarding the interaction (categories II and III). The scheme is shown in Table B-2.

This modified scheme facilitates the integration of toxicological and mechanistic data to support classification in an appropriate category. In common with the original scheme, it encourages the use of structure-activity information to support a classification. Because it is less prescriptive than the original BINWOE classification scheme, the modified scheme may require a greater degree of judgment in actual use.

Like the original method, the modified method does not take dose into account during the BINWOE determination, but rather during application of the algorithms (Section B.3.2).

B.3.2. MODIFIED INTERACTIONS-BASED HAZARD INDEX

The modified WOE method modifies each component's hazard quotient (where HQ_i is the hazard quotient of the i^{th} component) by the influences of all the other potentially interacting components, resulting in a hazard quotient modified for interactions (HQ_{i_I}). The interactions-modified hazard quotients are then summed to estimate the interactions-based hazard index (HI_I):

$$HQ_{i_I} = \sum_{i \neq j}^{n} HQ_i \, f_{j,i} \, M_{i,j}^{BINWOE_{i,j} \theta_{i,j}} \quad \textbf{(9)}$$

$$HI_I = \sum_{i=1}^{n} HQ_{i_I} \quad \textbf{(10)}$$

The overall process is shown in the following equation (EPA 1999). Some of the terms in equations 9-11 are modified slightly from those in the cited publications for consistency with the terms used in the original methodology.

$$HI_I = \sum_{i=1}^{n} \left(HQ_i \cdot \sum_{j \neq i}^{n} f_{j,i} \, M_{i,j}^{BINWOE_{i,j} \theta_{i,j}} \right) \quad \textbf{(11)}$$

Table B-2. Modified Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

		Default Weighting Factors	
		Direction	
Category	Description	Greater than additive	Less than additive
I.	The interaction has been shown to be relevant to human health effects and the direction of the interaction is unequivocal.	1.0	-1.0
II.	The direction of the interaction has been demonstrated *in vivo* in an appropriate animal model and the relevance to potential human health effects is likely.	0.75	-0.50
III.	An interaction in a particular direction is plausible but the evidence supporting the interaction and its relevance to human health effects is weak.	0.5	0.0
IV.	The assumption of additivity has been demonstrated or is accepted because the information is: A. Insufficient to determine the direction of any potential interaction. B. Insufficient to determine whether any interaction would occur. C. Adequate as evidence that no toxicologic interaction between the components is plausible.	0.0	0.0

*Adapted from EPA 1999

The term $f_{j,i}$ scales the interactions contribution of chemical j by its importance relative to all the other chemicals interacting with chemical i. The toxicological importance is represented by the hazard quotient:

$$f_{j,i} = \frac{HQ_j}{HI_{add} - HQ_i} \quad \textbf{(12)}$$

$M_{i,j}$ is the magnitude of the interaction, defined as an estimate of the maximum effect that chemical *j* has on the threshold or risk-specific dose (e.g., ED_{10}) of chemical *i*. When, as is often the case, data regarding the magnitude are not available, a default value of 5 is used, which is consistent with the upper end of the range of deviation from additivity shown by Smyth et al. (1969). The direction of the interaction is not incorporated into *M*, but rather is part of the term $BINWOE_{i,j}$, which is the BINWOE score. Positive values indicate the interaction is greater-than-additive, negative values indicate less-than-additive, and the value of zero indicates additivity. $M_{i,j}$, raised to the power of $BINWOE_{i,j} \bullet \theta_{i,j}$, functions as an uncertainty or modifying factor in the estimation of the interactions-based hazard quotients. The term $\theta_{i,j}$ reflects the degree to which components *i* and *j* are present in equitoxic amounts, based on the hazard quotients. This term is incorporated into the algorithm to account for the assumption that the greatest deviation from additivity will occur when both components in a binary mixture are present in equitoxic amounts (EPA 1999) . As discussed previously, this assumption is explicit in a model of a deviation from dose additivity proposed by Finney (1942, 1971). The measure of the deviation from equitoxic amounts is the ratio ($\theta_{i,j}$) of the geometric mean to the arithmetic mean of the hazard quotients:

$$\theta_{i,j} = \frac{(HQ_i \cdot HQ_j)^{0.5}}{(HQ_i + HQ_j)/2} \qquad \textbf{(13)}$$

As HQ_i approaches HQ_j, $\theta_{i,j}$ approaches 1, and as HQ_i and HQ_j deviate from each other, $\theta_{i,j}$ approaches 0. Thus, the term $\theta_{i,j}$ reflects how close to equitoxic are the two chemicals' doses. The value for of $\theta_{i,j}$ is the same (0.94) for two components with hazard quotients of 0.01 and 0.02, or 0.1 and 0.2, or 1 and 2.

B.3.3. STRENGTHS AND LIMITATIONS OF THE MODIFIED WOE METHOD

The modified WOE method may require more judgment in the determination of BINWOEs than does the original WOE method. The increased flexibility and the integration of toxicological and mechanistic information could lead to a more holistic assessment, but the flexibility also could lead to an erratic application of the methodology. Consistency of application has not been tested.

Although both WOE methods use BINWOE scores to modify an uncertainty (or magnitude) factor that can be based on the magnitude of the interactions, the original method focuses on a single uncertainty factor for the entire mixture, whereas the modified method focuses on individual magnitude factors (*M*) for the effect of each component on the toxicity of each other component. Thus, the potential advantage of the modified WOE method is that information on the magnitude of interactions can be applied directly to the hazard quotient of the chemical whose toxicity is affected. A default magnitude value of 5 is used

when data regarding magnitude are not available. This method is relatively new, and, as of this writing, has not been tested to determine whether toxicologists can apply it consistently and how well it predicts the toxicity of simple mixtures. It does appear to handle changing proportions of mixture components in a reasonable manner.

B.4. PRACTICAL CONSIDERATIONS FOR IMPLEMENTATION OF A WOE METHOD IN PUBLIC HEALTH ASSESSMENTS

The number of possible pairs in a mixture of N components is $(N^2-N)/2$. Thus a mixture of 4 chemicals has 6 possible pairs needing 12 BINWOEs, a mixture of 6 chemicals has 15 possible pairs needing 30 BINWOEs, and a mixture of 9 chemicals has (81-9)/2 = 36 possible pairs needing 72 BINWOEs. Obviously, the practicality of either WOE method may be an issue for mixtures with more than 4-5 components because of the large numbers of BINWOE determinations that would be required. If an algorithm is used, the calculations are fairly extensive.

Some ways of addressing this issue of practicality are as follows:

- Limit the use of the WOE method to those situations where clarification of the public health hazard is needed, such as sites where exposures to individual components are high enough, relative to health guidelines, that additivity and interactions may result in a significant health hazard.
- Focus the BINWOE effort on chemical pairs that frequently pose the above situation for ATSDR health assessments.
- Make BINWOE determinations available through an easily accessible and readily updated medium, such as the ATSDR website or Interaction Profiles.
- Further develop the patterns approach to analyzing and predicting interactions (Durkin et al., 1995) (see also Appendix A, Section A.3.3) as a potentially cost-effective means of generating BINWOEs.
- Develop a spreadsheet programmed with the appropriate equations to carry out the WOE calculations (if an appropriate algorithm is developed/fully evaluated/selected).

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APPENDIX C

THE INTEGRAL SEARCH SYSTEM FOR RANKING HAZARDS OF MIXTURES OF CARCINOGENS

C.1. INTRODUCTION

The Integral Search System (ISS) was designed to facilitate the use of interactions data in the component-based assessment of carcinogenic effects from exposure to chemical mixtures (DiCarlo and Woo 1994; Woo et al. 1994). An overview of this method was presented in the Guidance Manual (Section 2.3.6); some of that information will be repeated here as needed for understanding of the complete method, the details of which are presented in the following sections. The method also has been reviewed by Mumtaz et al. (1994) and EPA (1999).

Like the weight-of-evidence (WOE) methods (Appendix B), the ISS uses data for binary mixtures to predict the hazard of exposure to mixtures of three or more chemicals. The ISS is a software package that integrates three EPA and National Cancer Institute databases on binary interactions of carcinogens with other carcinogens (Arcos et al. 1988), with promoters (Rao et al. 1989) and inhibitors (Bagheri et al. 1988-89). A user's manual provides directions for using the software (Polansky and Woo 1994). The ISS calculates a weighting ratio that reflects the ratio of greater-than-additive to less-than-additive interactions for the components of a mixture. In addition, ISS can be used to estimate a level of concern based on the slope factors (potencies) of the components and the weighting ratio. Because the estimate of level of concern does not include a consideration of exposure level, its usefulness is limited.

C.2. WEIGHTING RATIO

The ISS computer program generates a list of all possible binary combinations of the mixture components. It then searches for interactions data for each pair and each category of interaction (synergism [syn], promotion [pro], antagonism [ant], and inhibition [inh]). A "name pair hit" (H_A) is tallied when information on a pair of components is located for any of these interaction categories. For each pair of components, the program registers only the first hit encountered for each interaction category. The total count of name pair hits for all component pairs is designated by, for example, $H_{A\,syn}$ for synergism.

For each pair with no name pair hits, the ISS searches for interactions between members of the structural or functional classes to which the components lacking data belong. Hits identified in this manner are called "class pair hits." The total number of class pair hits for each category of interaction is statistically adjusted in order to take into account the frequency and distribution of different interaction categories and the representativeness of the classes in ISS, and to insure that the inferred value will not exceed the value of a name pair hit, which is unity. The derivation of this adjustment procedure is highly complex, requiring eight pages of explanation in the software manual (Polansky and Woo 1994). The result is an "inferred class pair value" (H_B).

The name pair hits (H_A) and inferred class pair values (H_B) for each interaction category are then totaled as shown in the following example for synergism:

$$H_{syn} = H_{A\ syn} + H_{B\ syn} \quad \textbf{(1)}$$

The extent of hazard modification due to interactions among mixture components is estimated as a weighting ratio (WR):

$$WR = \frac{1 + (pH_{syn} + qH_{pro})}{1 + (rH_{ant} + sH_{inh})} \quad \textbf{(2)}$$

where p, q, r, and s are weighting factors for the effectiveness of the four types of interactions to modify the hazard of the mixture based on additivity. Based on their review of the interactions literature, Woo et al. (1994) consider the following to be reasonable default values: $p = 0.3$, $q = 0.7$, $r = 0.3$, and $s = 0.6$. These default values have been incorporated into the ISS program, but can be changed by the user.

The presence of the number one in both the numerator and denominator of the weighting ratio prevents the weighting ratio from reducing to zero when both H_{syn} and H_{pro} are zero, or from becoming infinity when both H_{ant} and H_{inh} are zero. When no interaction information is available or when the information for greater-than-additive interactions is equal to that for less-than-additive interactions, the weighting ratio is unity, and the hazard assessment is unchanged.

C.3. INHERENT CANCER HAZARD AND LEVEL OF CONCERN

Calculation of the inherent hazard, like the calculation of total cancer risk discussed in the Guidance Manual (Section 2.3.5), is based on the assumption of response additivity with a completely negative correlation of tolerances. The ISS program, however, does not include exposure concentration or dose as

part of the procedure. Instead, ISS calculates the inherent hazard as the sum of the cancer slope factors of the components, expressed in units of $(mmole/kg\text{-}day)^{-1}$. The sum is then converted by ISS to an exponent index, which is a linear scale of hazard indicators that approximately parallels the ranking of exponents of the slope factors (Table C-1).

Table C-1. Correspondence Among Slope Factors, Exponent Indexes, and Concern Levels*

Slope Factor $(mmole/kg/day)^{-1}$	Exponent Index	Concern Level
0 to $<5x10^{-5}$	0 to <1	Low
$5x10^{-5}$ to $<5x10^{-1}$	1 to <4	Marginal
$5x10^{-1}$ to $<5x10^{0}$	4 to <6	Low-moderate
$5x10^{0}$ to $<5x10^{1}$	6 to <8	Moderate
$5x10^{1}$ to $<5x10^{2}$	8 to <10	High-moderate
$5x10^{2}$ to $\approx 5x10^{7}$	10 to $\approx$14	High

*Adapted from DiCarlo and Woo (1994)

This correspondence table was developed for a set of 134 chemicals with known slope factors. The correspondence table constitutes an interface with structure-activity relationship (SAR) analysis, which is being used to provide a judgment regarding carcinogenic potential and a rough estimate of slope factor (as concern level) for data-poor chemicals through the computer program OncoLogic (DiCarlo and Woo 1994; Polansky and Woo 1994; Woo et al. 1995).

ISS multiplies the inherent hazard, in units of exponent index, by the weighting ratio (from Section C.2). The resulting weighted exponent index is then converted by ISS to a weighted total slope factor and to a corresponding concern level, ranging from low to high, as shown in the right column of Table C-1.

C.4. STRENGTHS AND LIMITATIONS

The obvious strengths of the ISS are that it performs the analyses automatically, and can be applied to mixtures with relatively large numbers of components, including components not presently included in the database, provided those components can be assigned to an appropriate class of chemicals within the database. The ISS does not, however, evaluate the relevance of the data to the anticipated exposure

scenario in the manner that the WOE method does. Nor does it provide an indication of the strength of the evidence for a particular interaction. A serious limitation of the ISS is that exposure levels are not taken into account during the procedure. As discussed in the Guidance Manual (Section 2.3.6), this limitation may be circumvented, at least in part, by restricting the use of this method to components whose exposures fall within a limited range of estimated risks or are considered toxicologically significant. The weighting ratio could then be used as an alternative weight-of-evidence score for interactions. Another serious limitation is that the class-class interaction ratings for pairs of chemicals with no data tend to dominate the score. ISS and OncoLogic are in use by EPA, but both are undergoing further review and development, which may address the limitation regarding the class-class interactions.

C.5. REFERENCES

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